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A modular process risk model structure for quantitative microbiological risk assessment and its application in an exposure assessment of *Bacillus cereus* in a REPFED

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Abstract

A modular process risk model (MPRM) methodology is presented as a tool for quantitative microbial risk assessment (QMRA). QMRA is increasingly popular to evaluate food safety and as a basis for risk management decisions. The MPRM provides a clear structure for transmission models of (complex) food pathways, that may involve the 'farm to table' trajectory. The MPRM methodology is illustrated in a case study, an exposure assessment of a sporeforming pathogen, *Bacillus cereus,* in a refrigerated processed food of extended durability (REPFED). This study is part of a European collaborative project. If contaminated with a psychrotrophic *B. cereus* strain, up to 6.5 % of the packages may contain more than the critical level of the pathogen at the moment the consumer takes the product from the fridge. This is, however, an uncertain estimate and its implications for public health are obscure. Some important gaps in knowledge are identified, for example the absence of growth and inactivation models that include variability, and useful quantitative models for sporulation, germination and spoilage. Also, the scarcity of data on consumer behavior concerning transport, storage and preparation is noted. Nevertheless, some risk mitigation strategies are suggested: decontamination of the ingredients that are added to the product, and improved temperature control of consumer fridge. Also, it is shown that a quality control at the end of industrial processing is highly insensitive. It was concluded that using MPRM is a promising approach, but that an improved interaction between risk assessment and microbiology is necessary.

Preface

This report describes research that is an integration of several projects. The methodology is part of the RIVM project 257851 'Exposure modelling of zoonotic agents in the animal production chain'. The exposure assessment is part of the project "Research on factors allowing a risk assessment of spore-forming bacteria in cooked chilled foods containing vegetables (RASP)". The RASP project has been carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT97-3159. It does not necessarily reflect its view and in no way anticipates the Commission's future policy in this area, which at RIVM is classified under project 149106 'Quantitative safety aspect of pathogens in food'.

Many people have contributed to the work outlined here. First of all, the participants of the RASP project. Of those, Antonio Martinez, Pablo Fernandez, Auri Fernandez, Frederic Carlin, Roy Moezelaar, Sonia Litman and Gary Barker are acknowledged for their specific contributions to the work described in this report. Especially the many contributions of Frederic Carlin, co-author of chapter 3, were indispensable.

The description of the food pathway, and hence the whole study, was only possible by the availability of the production process, provided by a food manufacturing company, which is referred to as 'company X' throughout this report. This company is gratefully acknowledged. Due to both identified and unidentified uncertainties*, the results in this report may in no way be related to the quality of any of their products*.

Several people at RIVM cooperated by their critical comments on (specific parts of) the research and earlier version of the report: Arie Havelaar, Eric Evers, Katsuhisa Takumi, Peter Teunis, Wout Slob and Frans van Leusden. They, too, are gratefully acknowledged.

Contents

Samenvatting

Dit rapport combineert de methodologische beschrijving van een raamwerk voor kwantitatieve risicoschatting (QMRA) met een concreet voorbeeld, een blootstellings-schatting van *Bacillus cereus* in een groenteproduct. QMRA wordt in toenemende mate toegepast ten behoeve van de voedselveiligheid en ter ondersteuning van daaraan gerelateerd risicomanagement, zowel door voedselproducenten als door de overheid. Het kan een wetenschappelijke basis bieden voor de besluitvorming op het gebied van voedselveiligheid ten behoeve van volksgezondheidsbeleid en in de internationale handel. De methodiek van QMRA is nog volop in ontwikkeling. In dit rapport wordt voorgesteld voor de blootstellingsschatting een "Modular Process Risk Model" (MPRM) te gebruiken.

Het MPRM (Hoofdstuk 2) biedt een heldere structuur voor transmissiemodellen van complexe 'voedselpaden', die zich kunnen uitstrekken over het hele traject van boerderij tot tafel. Een 'voedselpad' is gedefiniëerd als de rij van processen van r(a)uwe producten tot aan consumptie door de mens en omvat zowel de industriële bereiding, de detailhandel als de consument. In het MPRM wordt aan elke processtap in het voedselpad in principe één specifiek basisproces toegewezen: groei, inactivatie, mengen, opdelen, verwijderen of kruisbesmetting. De transmissie van micro-organismen door het gehele voedselpad is vervolgens te modelleren door voor elk van de basisprocessen een apart transmissiemodel op te stellen. Als een processtap zo ingewikkeld is dat er geen basisproces aan is toe te wijzen of als belangrijke parameters onbekend zijn, kan een 'black box' model gebruikt worden.

Het MPRM biedt een heldere structuur, niet alleen door het opdelen in basisprocessen, maar ook doordat in de opzet zeven opeenvolgende stappen worden beschreven, die doorlopen moeten worden om een blootstellingschatting vorm te geven. Het wordt daardoor eenvoudiger de modelaannames expliciet te benoemen. In het MPRM worden zoveel mogelijk mechanistische modellen gebruikt. In principe hangt het opstellen van een MPRM daarom niet af van de beschikbaarheid van gegevens. Gegevens zijn "slechts" noodzakelijk om tot een betrouwbare risicoschatting te komen, niet om het MPRM op te stellen. Het gebruik van tweede orde Monte Carlo modellen, waarin onderscheid gemaakt wordt tussen onzekerheid en variabiliteit, wordt aangeraden.

De MPRM methodiek wordt middels een concreet voorbeeld van een blootstellingsschatting uit de doeken gedaan (Hoofdstuk 3). Het voorbeeld betreft een sporenvormende pathogeen, *Bacillus cereus,* in een koelvers product, ofwel een "bewerkt, gekoeld levensmiddel met verlengde houdbaarheid" (REPFED): een pak met gepureerde broccoli. Het bijbehorende onderzoek maakte deel uit van een Europees samenwerkingsverband in een project over sporenvormende bacteriën in voedsel, afgekort tot RASP. Aan dit EU-project namen onderzoeksinstituten en levensmiddelenproducenten uit verschillende landen in Europa deel. Uit de blootstellingsschatting kan geconcludeerd worden dat consumenten door consumptie van het onderzochte product blootgesteld kunnen worden aan *B. cereus.* De mate van blootstelling is in hoge mate afhankelijk van het gedrag van de consument. Met de huidige

kennis (die ondermeer inhoudt dat er geen dosis-respons relatie beschikbaar is), is het niet mogelijk het bijbehorende risico te kwantificeren, of met enige zekerheid een uitspraak te doen omtrent eventuele gezondheidsrisico's die aan het onderzochte product verbonden zijn.
Zoals opgemerkt, is van *B. cereus* geen dosis-respons relatie bekend. Op grond van epidemiologisch onderzoek wordt een concentratie van 10^5 cfu/g vaak als een kritische waarde beschouwd. Geschat wordt dat, op het moment dat de consument het product uit de koelkast haalt, 6.5% van de pakken een concentratie *B. cereus* van meer dan 10^5 cfu/g bevat. Het betreft dan een psychrotrofe stam. Deze schatting is echter erg onzeker, en de consequenties van dit resultaat voor de volksgezondheid zijn vooralsnog niet in te schatten. Het is echter wel mogelijk maatregelen aan te wijzen, die de blootstelling aan *B. cereus* kunnen verlagen en daarmee het potentiële risico kunnen reduceren. De belangrijkste zijn decontaminatie van enkele ingrediënten die tijdens het productieproces aan het product worden toegevoegd, en een verbeterde beheersing van de koelkasttemperatuur bij de consument thuis. Steekproefsgewijze controle van producten aan het einde van de industriële productie is weinig gevoelig, want besmetting daar is een slechte voorspeller van het uiteindelijke risico voor de consument.
Om blootstellingsschatting van sporenvormende pathogenen goed te kunnen uitvoeren is het nodig dat er groei- en inactivatiemodellen ontwikkeld worden, waarin ook variabiliteit wordt meegenomen. Ook is er grote behoefte aan wiskundige modellen die sporenvorming en ontkieming van sporen beschrijven. Vervolgens zijn er dosis-respons gegevens en modellen nodig om de blootstellingsschatting uit te breiden tot risicoschatting. Bovendien zijn er veel meer gegevens nodig omtrent consumentengedrag. Het gaat daarbij met name om kennis over de omstandigheden van vervoer, opslag en bereiding van levensmiddelen.
Er kan geconcludeerd worden dat het MPRM raamwerk veelbelovend is. Om QMRA uit te voeren is een verbeterende interactie tussen twee geheel verschillende vakgebieden, microbiologie en risicoschatting, onontbeerlijk. Het MPRM raamwerk zal hierbij in de toekomst behulpzaam kunnen zijn.

Summary

This report combines the methodological description of a framework for quantitative microbial risk assessment (QMRA) with a case study of *Bacillus cereus* in a vegetable product. QMRA is increasingly popular to evaluate food safety and as a basis for risk management decisions, both by food manufacturers and public health authorities. It may serve as a scientific basis for food safety issues for public health policy and in international trade. The methodology QMRA is however not well established. Therefore a Modular Process Risk Model (MPRM) methodology is presented here as a tool for exposure assessment.

The MPRM (chapter 2) provides a clear structure for transmission models of (complex) food pathways, that may involve the 'farm to table' trajectory. The food pathway is the line of processes from the raw product(s) until human consumption, that includes both the industrial processing and the retail and consumer handling. Essentially, in the MPRM each processing step in the food pathway is identified as one of six basic processes: growth, inactivation, mixing, partitioning, removal and cross contamination. The transmission of microorganisms through the food pathway can be modelled by formulating models of the transmission through each of these basic processes. If the transmission is too complex or if essential parameters are unknown, a 'black box' model may be used.

The MPRM has the advantage that is has a clear structure, not only by the identification of basic processes, but also by the proposing seven consecutive steps to perform an exposure assessment. This simplifies the explicit statement of the modelling assumptions. Next, MPRM uses mechanistic models as much as possible. In principle, constructing an MPRM does not depend on the availability of data. Data are 'only' relevant to come to a reliable risk estimate, using the MPRM, not for the modelling itself. The use of second order Monte Carlo models, separating uncertainty and variability, is promoted.

The MPRM methodology is illustrated in a case study, an exposure assessment of a sporeforming pathogen, *Bacillus cereus,* in a refrigerated processed food of extended durability (REPFED): a package of broccoli puree (Chapter 3). This study is part of a European collaborative project on sporeforming bacteria (abbreviated as RASP), involving research institutes and food companies throughout Europe. It was found that consumers may be exposed to *B. cereus* by consumption of the products. The level of exposure is highly influenced by the consumer behaviour. With the present knowledge (which is, among others, characterised by the lack of dose response information), it was not possible to quantify the risk, or to draw any 'certain' conclusions on the risk of the product.

At the moment there is no dose-response relationship available for *B. cereus*. Based on epidemiological studies, a concentration of 10^5 cfu/g is generally considered as a critical value. As an estimate, at the moment the consumer takes the product form the refrigerator there may be a probability up to 6.5% of dealing with a pack that contains more than 10^5 cfu/g, if contaminated with a psychrotrophic *B. cereus* strain. This is, however, an uncertain estimate and its implications for public health are obscure. Nevertheless, some promising risk

mitigation strategies can be identified, which will effectively lower the exposure to *B. cereus*. The most obvious ones are decontamination of some ingredients added during the production of the product and improved temperature control of consumer refrigerators. Controlling for food safety at the end of the industrial process by taking random samples there appears to be a bad predictor for food safety risk for the consumer.

It is concluded that exposure assessment of sporulating pathogens needs the development of growth and inactivation models that include variability and quantitative models for sporulation and germination. The development of dose response models is necessary to extend the exposure assessment to a risk assessment. Also, the scarcity of data on consumer behaviour concerning transport, storage and preparation is noted.

It was concluded that using MPRM is a promising approach, but that an improved interaction between risk assessment and microbiology is necessary. MPRM will be extendedly developed and applied in other QMRA's in the near future.

1. Introduction

1.1 Outline

Quantitative microbiological risk assessment (QMRA) modelling is increasingly used as a tool to evaluate food related health risks. Recently, an increasing number of papers has appeared that describe QMRA studies, for a range of micro-organisms and food products (Cassin et al. 1998a, Bemrah et al. 1998, Whiting and Buchanan 1997). However, as discussed below, the methodology of QMRA modelling is not yet established. Different approaches have been used so far, and several protocols and guidelines have been proposed. (CODEX Alimentarius Commission 1998, McNab 1998, Marks et al. 1998, Cassin et al. 1998a, ILSI 2000).
In this report we propose the use of modular process risk models (MPRMs) as a tool for risk assessment modelling. An important characteristic of this approach is the modular structure, that splits the food pathway into processing steps that describe one of six basic processes: growth, inactivation, partitioning, mixing, removal and cross contamination. In theory, once the modelling of these basic processes is established, any food pathway can be modelled when it is described as a sequence of consecutive basic processes.
The description of MPRM as a quantitative microbial risk assessment modelling framework is illustrated with an example: an exposure assessment of *B. cereus* in broccoli puree. The model constructed in this example is the result of a collaborative European research project on sporeforming pathogens in cooked and chilled vegetable products, abbreviated as 'RASP' (Carlin et al. 2000a).
The main objective of this report is to describe and illustrate a methodology, and examine its utility. With the example, we additionally identify some important gaps in knowledge and we are able to suggest some risk mitigation strategies for the specific product of concern.

1.2 QMRA methodology

The CODEX Alimentarius Commission (1998) guidelines for the conduct of microbial risk assessment gave a list of principles and definitions, but did not present a modelling methodology. McNab (1998) presented a framework for a Monte Carlo model of a generic food system. This framework describes the whole 'farm to table' pathway, and the additional health risks with biological and economical impact. However, it makes some disputable explicit choices for sub-models, and does not consider second order Monte Carlo modelling. As it has no modular structure, it is difficult to use as a general framework. Marks et al. (1998) used the term 'Dynamical flow tree' (DFT) modelling for their strategy. It incorporates the dynamics of microbial growth for microbial risk assessment. The DFT process is based on data and formal statistical inference or extrapolation from the available data. Cassin et al. (1998a) introduced the term process risk model (PRM) to describe the

integration and application of QRA methodology with scenario analysis and predictive microbiology. The emphasis of the PRM is to apply QRA as a tool that can be used to identify intervention procedures that might mitigate the risk, not the quantitative estimate of risk per se.

Van Gerwen et al. (2000) propose the use of stepwise quantitative risk assessment as a tool for characterisation of microbiological food safety. Their approach allows to tackle the main problems first, before focussing on less relevant topics. The first step is a broad, qualitative assessment, the second step a rough quantitative assessment and the third step a quantitative risk assessment using stochastic modelling. This approach is particularly useful in the development of food production processes and for pointing out crucial processing steps in the food pathway.

As a general framework, we propose a modular process risk model (MPRM) structure for quantitative microbial risk assessment. For a large part, it resembles the approach of Cassin et al. (1998a). Like their PRM, the model focuses on the transmission of the hazard along the food pathway, by describing the changes in the prevalence and the concentration over each of the processing steps. This food pathway includes production, processing, distribution, handling and consumption (Cassin et al. 1998b). With the model, the effects of 'alternative scenario's', that is the effects of specified risk mitigation strategies or presumed changes in food processing or consumer behavior, can be assessed. However, our approach differs with that of Cassin *et al.* in two respects. First, it has a modular structure. It states that the transmission of the hazard through the food pathway can be regarded as a series of basic processes. The model structure is determined by this series of basic processes, as will be explained below. Unlike the DFT process (Marks et al. 1998) it is not based on the availability of data, but on knowledge of the food pathway processes. Second, we propose to use second order Monte Carlo modelling, that is the separation of uncertainty and variability in stochastic modelling. In this context, 'uncertainty' represents the lack of perfect knowledge of the parameter value, which may be reduced by further measurements. 'Variability', on the other hand, represents a true heterogeneity of the population that is a consequence of the physical system and irreducible by additional measurements (Murphy 1998, Anderson and Hattis 1999). Previously we have shown that neglecting the difference between these two may lead to improper quantitative risk estimates (Nauta 2000b). Without applying second order modelling dissimilar probability distributions are easily messed up, resulting in non-informative output distributions.

The MPRM may be used in any QMRA study, that is for industrial food processing and 'farm to table' risk assessment models. The focus is on modelling the food pathway and therefore on exposure assessment, that is the assessment of the dose of micro-organisms present in the food at the moment of consumption. Dose response modelling falls outside the scope of this report.

1.3 The example: *B. cereus* in broccoli puree

1.3.1 The RASP project

The example of the MPRM constructed in this report concerns *B. cereus* in broccoli puree packages. This example originates from a EU collaborative project entitled 'Research on factors allowing a risk assessment of spore-forming pathogenic bacteria in cooked chilled foods containing vegetables (FAIR CT97-3159)', abbreviated as 'RASP' (Carlin et al. 2000a). In this four year project (October 1996-September 2000) food microbiologists and risk assessors from INRA (France), IFR (UK), Nottingham University (UK), IATA (Spain), ATO (Netherlands) and RIVM (Netherlands) collaborated, with some food manufacturers from Spain, France and Italy, and SYNAFAP, a food processor association from France. As can be read from the project title, it concentrates on risk assessment of spore forming bacteria (SFB) in refrigerated processed foods of extended durability (abbreviated as REPFEDs), a new generation of cooked chilled foods. These REPFEDs follow the new consumer demands for more convenient, fresher, more natural foods of high organoleptic quality, which implies lighter preservation methods. REPFEDs are generally processed with mild heat-treatments and rely on refrigeration for preservation. Survival and outgrowth of SFB on REPFEDs may lead to food poisoning, and may therefore be an increasing threat to public health.
The project consisted of both experimental research and mathematical modelling on *Bacillus cereus* and *Clostridium botulinum* from vegetable products. The concentration of experts within the project allowed the construction some risk assessment models, using both the 'Monte Carlo'-technique, and Bayesian networks. This report concentrates on the '*B. cereus* in broccoli puree' MPRM, using the Monte Carlo technique. This example illustrates the use of most of the different modules as explained in the methodology section.
The RASP project has been structures around the guidelines as given in the Codex Alimentarius(1998). These guidelines state that a risk assessment should consist of a hazard identification, a hazard characterization (including a dose response assessment), an exposure assessment and risk characterization. As stated above, we concentrate on the exposure assessment. The results of RASP concerning the other parts of the risk assessment are discussed elsewhere (Carlin et al. 2000a, F. Carlin, G. Barker and others, *unpublished*).

1.3.2 *B. cereus* in broccoli puree

The choice for the hazard / product combination *Bacillus cereus* / broccoli puree is based on the results and expertise in the RASP project. With *Clostridium botulinum*, *B. cereus* is the 'high risk' hazard identified in the hazard identification (Van Leusden, *unpublished*). *B. cereus* is a well known pathogen (e.g. Notermans and Batt 1998, Granum and Lund 1997, Choma et al. 2000). An important characteristic, relevant for risk assessment, is that it is a sporeforming pathogen and may therefore be resistant to heat. The actual hazard is not the bacterial cell, but its toxin. *B. cereus* can form two types of toxin:

(1) an emetic toxin, which is preformed in the food, mainly found in rice products, and therefore not relevant in broccoli puree, and
(2) an enterotoxin, which is formed after outgrowth of the bacteria in the intestine and which is sensitive to heat and acidity. This is the toxin of interest in this study.
Unfortunately a dose response relationship, relating either toxin dose or pathogen dose to health effects, is unavailable. As discussed by Notermans et al. (1997), a level of 10^5 organisms per gram is generally considered as critical limit for acceptance of a food product. The absence of a dose respons relationship is the main reason that the risk assessment in the RASP project is restricted to an exposure assessment.
The variability in growth and inactivation characteristics between *B. cereus* strains may be large. A major type difference is that between psychrotrophic strains and mesophilic strains. The psychrotrophic strains are considered to be the major hazard in REPFEDs, as they are able to grow at low temperatures. However, mesophilic strains may be more heat resistant (Dufrenne et al. 1995), and might therefore be a hazard as a consequence of mild heating and severe temperature abuse.
The choice for broccoli puree as the product of interest is mainly based on the availability of data on its production process, some contamination data and some information on growth in broccoli broth.

2. QMRA methodology : A modular process risk model (MPRM) structure

2.1 Setting up a QMRA

According to the CODEX Alimentarius Commission (1998) guidelines, risk analysis is the combination of risk management, risk assessment and risk communication. The aim of the risk assessment should be defined by the risk management. Next, the risk assessor needs several definitions before he can start the risk assessment. This is illustrated in Fig 2-1.

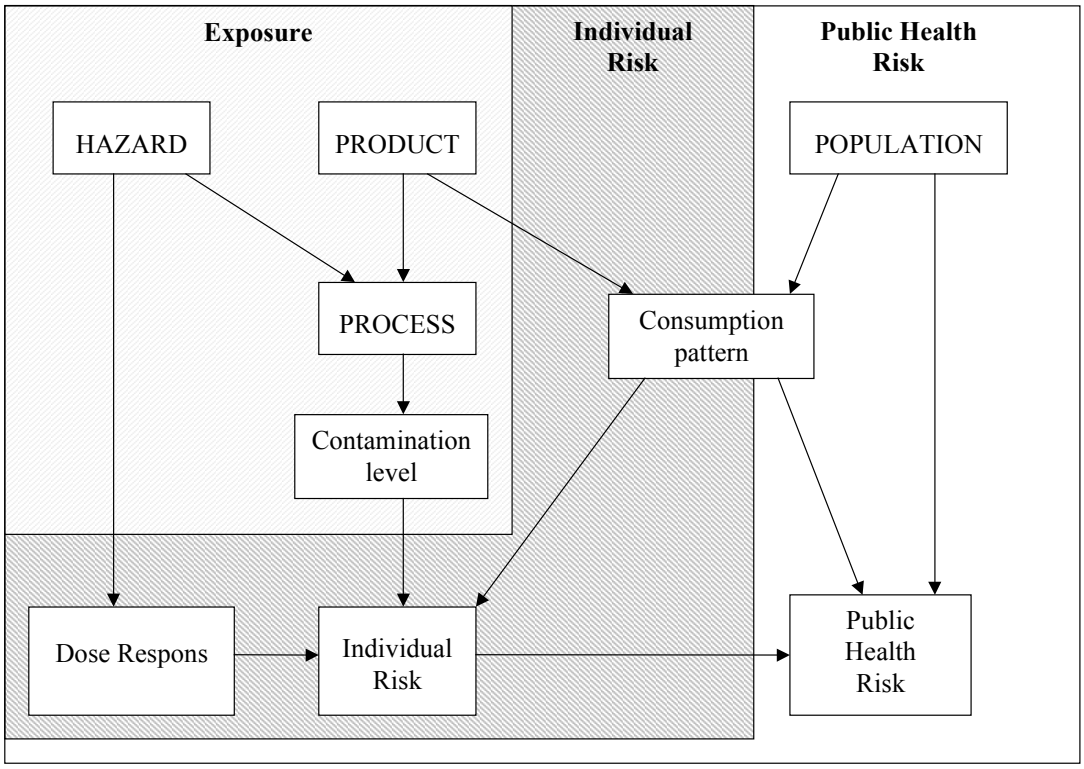

Figure 2-1 A risk assessment scheme. The boxes with capitals hold the items to be defined before setting up the risk assessment. If risk assessment is restricted to either exposure assessment or assessment of individual risk, the scheme is reduced to the shaded areas. According to this scheme, exposure assessment is defined as the assessment of the level of contamination in a food product, independent of consumption patterns. Different assumptions or information on the consumption pattern is needed for assessment of individual risk and public health risk.

If the aim of the risk assessment is to assess a public health risk of consumption of a food product, definitions are needed of the hazard, the product, the population and the food pathway. Of these, the risk manager should at least provide the product and the population. It may be the task of the risk assessor to identify the hazard(s) and to define the food pathway, but these may also be part of the statement of purpose provided by the risk manager. In

microbiological risk assessment the hazard is the microorganism of concern (although the actual hazard may be a toxin it produces). The product is the food stuff consumed, and the food pathway is a description of the route of transmission of the hazard through the production process of the product until the moment of consumption. With these three a risk assessor should be able to assess the exposure, and, if a dose response relation is known, to assess the individual risk of consumption of the product. To assess the public health risk, one needs a definition of 'public', that is the population of concern. Consumption data of the product by that population are then needed to assess the consumption pattern. This has to be incorporated in the exposure assessment and combined with the dose response relation, to finalise the public health risk assessment.
As can be seen from Fig 2-1, definition of hazard, product and food pathway are necessary for exposure assessment. If the assessment has to be extended to some assessment of individual risk, one additionally needs a dose response relation, and if it has to be extended to assessment of the public health risk, one also needs a definition of the population.
If information on any of the requested items is lacking, it is possible to do a risk assessment, by making assumptions on e.g. the food pathway or consumption patterns of the population. It is important that these assumptions are clearly reported after finishing the risk assessment. Making assumptions should not be a problem in risk assessment, as long as they are explicitly reported, and as long as their impact is thoroughly discussed.

2.2 Setting up an MPRM

The modular process risk model (MPRM) is a tool for quantitative exposure assessment modelling. In general, the aim of the process model is to describe the transmission of the hazard along the food pathway, taking into account the variability and uncertainty attending this transmission. For this purpose, the food pathway is split up smaller steps, the modules. For each module, we are interested in the input-output relation for the number of cells per unit of product, N, and the fraction of contaminated units, the prevalence P. By treating both N and P as uncertain and variable throughout the model, we will be able to assess the uncertainty and variability in the final exposure, and thus the uncertainty in the final risk estimate.
Essentially, this input-output relationship can be obtained either by observation (direct in the production process (surveillance) or indirect in an experimental simulation (challenge testing)) or by mathematical modelling (see e.g. Notermans et al. 1998). The first method has the advantage that it is process-, product- and micro-organism specific, but the disadvantage that it is usually time consuming and expensive. The second method has the advantage that it is quick and cheap, but the disadvantage that it may be imprecise and not specific for the process studied.
Here we focus on the second method, mathematical modelling. We propose an approach for the exposure assessment in a QMRA which holds the following steps:

1) Define the statement of purpose, the (microbial) hazard and the food product. Consider which are the alternative scenario's (either risk mitigation strategies, or potential changes is the process) that are to be evaluated with the model.

2) Give a description of the food pathway. Processing steps that involve potential alternative scenario's may need a more detailed description than processing steps that will remain unchanged.

3) Build the MPRM model structure, by splitting up the food pathway into small processing steps (modules). In principle, each module refers to one of six basic processes, as explained in detail below. If a processing step is to complex or if essential parameters are unknown, and the processing step cannot be assigned to any of the basic processes, it can be considered as a 'black box' process.

4) Collect the available data and expert opinion, according to the model structure developed.

5) Select the model to use for each module, on the basis of the statement of purpose, process knowledge, data availability and the alternative scenario's considered.

6) Implement the available data into the model. For each processing step, select the specific model to use. The use of mechanistic models is preferred, but only use complex models when this is necessary for evaluating alternative scenario's and when the availability of data permits it.

7) Perform an exposure assessment.

2.3 Modelling

2.3.1 Monte Carlo

Monte Carlo simulation is a technique widely used in quantitative risk assessment. It allows working with stochastic models and to dealing with variability and uncertainty, whilst simulating the transmission of the hazard along the food pathway. Currently we use @Risk software for this purpose (Pallisade, Newfield, USA). @Risk can be used as an add-on to an Excel spreadsheet, and is therefore relatively easy to use.
A Monte Carlo simulation program runs a large number of iterations. In principle, each iteration represents one potential true event of transmission along the food pathway. A random (usually Latin Hypercube) sample is drawn from each of the probability distributions used as input for the model parameters. The model output is then again a probability distribution, that allows us to evaluate 'probabilities' of events, and therefore to evaluate risks. It is in all instances important to realise how an 'event of transmission' is defined and

what the input probability distributions represent. An ‘event of transmission’ may be the ‘one batch’ production of one food manufacturing site, the per day production of a particular food stuff in a region, or the yearly consumed quantity in a country. In principle all input distributions should depict probability distributions over the same ‘events of transmission’. If, for example, one distribution describes the variability per year and another the variability per day, these distributions cannot be mixed up without thorough consideration.
Monte Carlo simulation has the advantage that it widely applicable, comprehensible and relatively easy to use. Important disadvantages of the Monte Carlo method are that it needs precise probability distributions for all input parameters (see below), may require an enormous number of iterations in complex models, is difficult to use when modelling (non-linear) dependencies and may not be an adequate tool when rare events have a major effect on the final result. When performing a Monte Carlo in a spreadsheet, one occasionally has to use some approximations, to restrict the number of cells and to keep the model running. These approximations are sometimes inevitable, but always have to be handled with care.

2.3.1.1 Choosing a probability distribution.

Monte Carlo modelling needs a precise definition of the probability distributions of the input parameters. The choice of the ‘best’ probability distribution is not always straightforward. Criteria for choosing a distribution are usually natural characteristics of the parameter and the process modelled, or the quality of the fit of a distribution to data. Overviews of different probability distributions that can be used are widely available (Vose 1998, Vose 2000). The list below is restricted to a very short description of some frequently used distributions with their most important characteristics in the context of risk assessment and Monte Carlo modelling. This description is neither precise, nor complete.

2.3.1.1.1 Binomial

The binomial Bin(n,p) distribution describes the probability of x successes in n trials, when the probability of success is known to be p. It can be considered as ‘the mother of all probability distributions’. To be used when n is relatively small, and p nor 1-p is close to zero.

2.3.1.1.2 Poisson

The Poisson(λ) distribution describes the probability of x ‘rare’ events when the expected number of events is λ. The Poisson distribution is related to the Binomial by $\lambda = np$. The binomial distribution is approximated by Poisson when n is large (say $n>50$) and p is small (say $np<5$).

2.3.1.1.3 Normal

The Normal N(μ,σ) distribution is a continuous distribution characterised by the mean μ and standard deviation σ. The Normal distribution is related to the Binomial by $\mu = np$ and $\sigma = \sqrt{np(1-p)}$. The binomial distribution is approximated by Normal when n is large (say $np>$

$3\sqrt{np(1-p)}$). The central limit theorem states that the sum of random variables with the same distribution approximately has a normal distribution. Many things in nature have a normal distribution. In many statistic tests it is assumed that the variables have a normal distribution.

2.3.1.1.4 Lognormal

In the lognormal distribution the logarithms of the parameter considered have a normal distribution. It has a lower limit zero, and a long tail. It is a convenient distribution because a log transformation allows one to treat multiplications as sums. It is important in (microbiological) risk assessment, because micro-organisms usually grow and die exponentially, and their numbers can therefore be considered as lognormally distributed. In general, many things in biology follow a lognormal distribution (Slob 1994).

2.3.1.1.5 Beta

The Beta(α,β) distribution is very flexible and well suited to describe the probability distribution of a probability, as it is bounded between 0 and 1. It can be used to express the uncertainty of the probability p in a binomial process when the number of successes s and the number of trials n is known. In that case, (assuming a uniform prior) p has a Beta(s+1, n-s+1) distribution.

2.3.1.1.6 Gamma (and exponential)

The Gamma(α,β) distribution is another continuous distribution with lower limit zero and no upper limit. It describes for example the probability distribution of the amount of time that lapses before α rare events happen, given a mean time between events β. If $\alpha = 1$ (so if the time until a single event is of interest) the Gamma distribution is equal to the 'exponential distribution'. An attractive characteristic of the Gamma distribution is that the sum of k random variables with a Gamma(α,β) distribution is Gamma ($k\alpha$,β) distributed.

2.3.1.1.7 BetaPert

The BetaPert(min, mod, max) distribution (Vose 2000) is derived from the Beta distribution. It is well suited to describe the uncertainty of a parameter assessed by expert opinion. When the minimum, most likely and maximum value of a parameter are assessed the BetaPert can be used to represent its distribution. The same can be done with a Triangle(min, mod, max) distribution, but this has the disadvantage that it has a rigid shape with more weight in the tails.

2.3.1.2 Second order modelling: uncertainty and variability

As shown elsewhere (Nauta 2000b), separation of uncertainty and variability may be important in risk assessment modelling. 'Uncertainty' represents the lack of perfect knowledge of the parameter value, which may be reduced by further measurements. 'Variability', on the other hand, represents a true heterogeneity of the population that is a consequence of the physical system and irreducible by additional measurements (Murphy

1998, Anderson and Hattis 1999). At the moment, so called second order models (models that make the separation of the two) are scarcely produced in microbial risk assessment. The result is that the probability distributions of the final risk are a mixture of uncertainty and variability, and may give a wrong insight in the final risk.
The most important thing in this context is to make clear what the applied probability distributions describe. Variability can be variability in time, space, population(s), products etc. By explicitly identifying what the variability stands for, these things cannot be mixed up.
A probability distribution describing variability can be uncertain, if the variability is not known exactly. Uncertainty can be very difficult to quantify. In that case the options are to neglect uncertainty and make qualitative statements on the uncertainty in the conclusions, or to assess the uncertainty throughout the risk assessment for *all* uncertain parameters by expert opinion or otherwise. To assess the uncertainty in the risk estimate, incorporating uncertainty for some uncertain parameters but neglecting it for others, is not a meaningful strategy. It may however be useful if the purpose is to assess the relative decrease in uncertainty of the final risk estimate by additional research on one specific risk factor.

2.3.2 Basic processes

The most important element of the MPRM approach is that we identify six basic processes, which are the backbone of every process risk model considered. These basic processes are the six fundamental events that may affect the transmission of any microbial hazard in any food process. There are two ‘microbial’ basic processes, growth and inactivation, and four ‘food handling’ processes, mixing and partitioning of the food matrix, removal of a part of the units, and cross contamination. The ‘microbial’ processes strongly depend on the characteristics of the microbial hazard, as the effects of environmental conditions on growth and inactivation differ per species (and even per strain). Essentially, the effects of the ‘food handling processes’ are determined by the food handling process characteristics only. Apart from aspects like ‘stickiness’ and cell clumping, which affect the distribution over the food matrix and may be different for different micro-organisms, they have the same effect on any hazard.
For each basic process a variety of models can be applied. The selected model for a certain module should be as simple as possible. Model selection will depend on the statement of purpose, process knowledge, data availability and the alternative scenario’s considered. If a process is well described, for example when process time, acidity and temperature are known, but microbiological data are scarce, one may use a predictive microbiology model to quantify growth or inactivation. If, on the other hand, data like microbiological counts before and after the process are available, but process parameters are not well known, it may be better to use a simple model relating the count data. If the process will remain unchanged, that if it is not part of any alternative scenario that changes the process, it is sufficient to have a simple description of the input-output relationship, without the involvement of any other parameters. (In that case it may, however, be necessary to use a complex model to derive this

relationship.) If, on the other hand, an alternative scenario changes the transmission of the hazard through the module, the effects of this scenario has to be part of the model.
The qualitative impact on the transmission of each of these basic processes is given in Table 2-1. Note that the columns in this table do not depict the concentration, but the total number of organisms (*N*) and the 'unit size'. The 'unit' is a physically separated quantity of product in the process, like for example a broccoli trunk, a carcass, a package of ground beef, a milk tank or a bottle of milk. The prevalence (*P*) in the table refers to the fraction of contaminated units. We prefer to use the number of organisms per unit and not concentrations. Doing so, we use only whole numbers in our calculations, which prevents the possibility of accidentally using 'fractions of individual cells'. As a consequence of this approach, it is essential to precisely define the unit size.
Although it should be possible to allocate a basic process to each processing step in a food production process, this may either be difficult or unnecessary in practice. Therefore some (consecutive) processes are better regarded as 'black boxes'. These processes may be very complicated and/or obscure. When there are no alternative scenario's considered that directly affect such a process, detailed modelling is not necessary. In that case we propose a linear 'black box' model.

Table 2-1 Basic processes of the MPRM and their qualitative effect on the prevalence (*P*), the number of organism in all units evaluated in one simulation run (N_{tot}) and the unit size. '=' : no effect,; '+': an increase; '-':a decrease.

	effect on P the fraction of contaminated units	effect on N_{tot} the total number of cells over all units	Effect on unit size
Growth	=	+	=
Inactivation	-	-	=
Mixing	+	=	+
Partitioning	-	=	-
Removal	-	-	=
Cross-contamination	+	= / +	=

How the transmission can be modelled quantitatively in each of the basic processes and with the 'black box' model is discussed below. The aim of the modelling of each of the basic processes is to describe the change in prevalence and number of micro-organisms per (contaminated) unit per processing step. The new prevalence (at the end of the basic process) is given by *P'*, and the new number or micro-organisms is given by *N'*. A change in unit size is usually defined in the food pathway description. *P'* and *N'* will be a function of *N*, *P* and the unit size, and possibly of some process parameters.

Note N and P, and possibly the unit size, are usually not fixed numbers, but stochastic variables that may be variable and uncertain. The prevalence P is a characteristic of all units at a step in the food pathway together. The number of micro-organisms N per unit may vary per unit.

2.3.2.1 Growth

The growth process is typical for microbial risk assessment. It is a variable, uncertain and complicating process. As can be seen from Table 2-1, growth gives an increase in both prevalence and number of micro-organisms and is therefore a particular risk increasing process. Due to growth, products that are contaminated at a low level and therefore are considered safe at a certain moment of time, may become unsafe later.
Growth is defined here as an increase in the number of cells N per unit. It does not affect the prevalence.
In general a growth model has the structure

$$\log (N_{out}) = \log (N_{in}) + f(.) \qquad (2.1)$$

with N_{in} the number of cells at the beginning of the process, N_{out} the number of cells at the end of the process and $f(.)$ an (increasing, positive) growth function. This growth function can have many shapes, which are widely discussed in literature (McMeekin et al. 1993, Whiting 1995, Baranyi and Roberts 1995, Van Gerwen and Zwietering 1998). For example, for exponential growth $f(t) = \mu t$ (with t is time and μ is the specific growth rate), and when using the Gompertz equation $f(t) = a \exp[-\exp(b-c\ t)]$, with parameters a, b and c.
As stated above, the selection of the 'best' model depends on of the statement of purpose, process knowledge and data availability. If, for example, a change in the temperature regime is considered, the model will need incorporation of 'temperature' as a parameter. Which specific 'secondary growth model' to select for this purpose, will depend on knowledge and the availability of data.
It is important to realise that a quantitative microbiological risk assessment growth prediction has usually different demands than a 'traditional' predictive food microbiology growth model prediction. Whereas the latter is used to come to a growth curve prediction, that is a series of point estimates of growth for a time series, in a risk assessment model, especially for a controlled food production process, one usually has to predict both variability and uncertainty in growth after a fixed time period (see Fig 2-2). Predictive microbiology models are not developed for this purpose, and therefore one has to be careful when applying them in a quantitative risk assessment study (Nauta 2000a, Nauta and Dufrenne 1999).

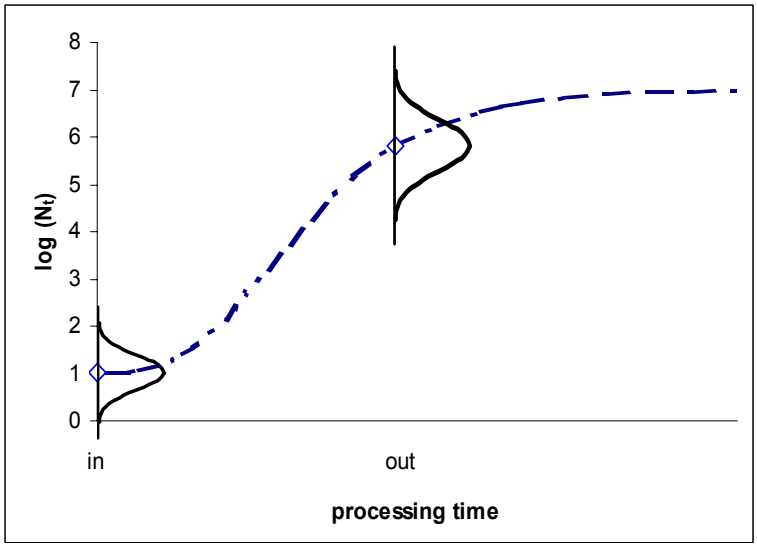

Figure 2-2 Growth is the increase in population size, given as log(N_t), as a function of time. Predictive microbiology models typically predict a growth curve, as given by the dashed line. In these models growth is considered as a function of time and the model yields a point estimate at any point of time *t*. In contrast, in QMRA we need a model that relates the probability distribution of the population size at the end of a process (out) to the probability distribution of the initial population size (in). Here, the end of the process may be at a fixed point in time. The probability distributions given by the 'bell curves' represent uncertainty and/or variability in population sizes N_{in} and N_{out}.

At the moment, to our knowledge, there are no predictive models available that are specifically designed to be used in a QMRA. Therefore, the best option is to use one of the available predictive microbiology models. Some of the frequently used models are response surface models based on data fitting, which are (commercially) available as computer software, like the Food MicroModel (Leatherhead Surrey, UK) and the Pathogen Modeling Program (PMP) (USDA, 1998). Another approach, primarily based on the principles of growth kinetics, allows a prediction if some of the growth characteristics (like the minimum and maximum temperature of growth) of the microorganism are known (Wijtzes et al. 1995, Zwietering et al. 1996).

It is not possible to say which model is the best choice in general. The PMP has the advantage that it is freely available at the internet and that it gives a confidence interval. It holds the problem that it is not clear whether this interval represents all of either variability or uncertainty or both. Also, the model prediction cannot easily be implemented in a risk assessment spreadsheet. Modelling according to the gamma concept (Wijtzes et al. 1995, Zwietering et al. 1996) has the advantage that it is a general model and can be easily applied to come to point estimates, but it gives no prediction of either uncertainty or variability.

In general it can be stated that the important thing is not which model you use, but that you use it properly. If the QMRA includes uncertainty assessment, uncertainty and variability should be included in the growth prediction too. If the uncertainty and variability of the growth prediction are not known (which is usually the case), it is better to assess them by

expert opinion, then to neglect them, as this might result in an improper uncertainty interval around the final risk estimate. If different models are available which yield different predictions, this may be implemented as 'model uncertainty' into the model.
Although the variability in growth of micro-organisms will often be unclear, a method is available to predict the variability in growth, which is particularly relevant when the number of cells is low. This method addresses the variability of growth of a single strain and is derived from birth-process theory (Marks and Coleman, *unpublished*). It is shown that when there is a fixed probability over time that any cell will divide in two, the increase I in the number of cells N will have a negative binomial distribution with parameters $s= N_0$ and $p = e^{-\mu t}$ and

$$E(I) = N_0(e^{\mu t}-1) \quad (2.2)$$
$$var(I) = N_0 e^{\mu t}(e^{\mu t}-1) \quad (2.3)$$

Assuming exponential growth, $E(N_t) = N_0 e^{\mu t}$, it can be derived that

$$N_t \sim N_0 + \text{Negbin}(N_0 , N_0 /E(N_t)). \quad (2.4)$$

Here we propose to use this equation as an approximation for the variability in growth for any growth model, so for any $N_{in} = N_0$ and $N_{out} = N_t$.
As the negative binomial distribution approximates the normal distribution when the mean is larger than about 4 times the standard deviation, the normal distribution can be used when $E(N_{out})$ is large, that is when $E(N_{out})>N_{in}^2/(N_{in}-16)$:

$$N_{out} \sim \text{Normal}(E(N_{out}), \sqrt{(E(N_{out})(E(N_{out})-N_{in})/N_{in})}). \quad (2.5)$$

It may be necessary to use this approximation for large values of N in a spreadsheet program.

2.3.2.2 Inactivation

Microbial inactivation is the opposite of microbial growth. It is characterised by a decrease in the number of organisms per unit N. If the inactivation results in a decrease to zero living cells, the prevalence will decrease too.
The general formula for modelling inactivation is similar to equation (2.1)

$$\log (N_{out}) = \log (N_{in}) - g(.) \quad (2.6)$$

with $g(.)$ an increasing (!) inactivation function. As for growth, many inactivation models are available (e.g.Van Gerwen and Zwietering 1998, Xiong et al. 1999). The most frequently used inactivation process is heating and the most frequently used inactivation model is the Bigelow model, in which inactivation rate is a function of temperature. It is linear in time (t)

and has the shape $g(t) = t/D$, when the D-value (the decimal reduction value) at the process conditions is known. When D_{ref} , the D-value at temperature T_{ref}, and the z-value are known, the same inactivation function holds at temperature T with

$$\log(D_T) = (T-T_{ref})/z + \log(D_{ref}) \quad (2.7)$$

Many of the aspects discussed for growth also apply for inactivation. Inactivation models are not developed to predict the uncertainty and variability around the final estimate. Confidence intervals may be derived from experimental data, but it is not straightforward whether to interpret this as uncertainty or variability. As for growth, an important aspect here is the differences that may exist between strains of a species, and even differences within a strain, due to adaptation (Nauta and Dufrenne 1999).
As for growth, the variability in inactivation can be predicted when the number of cells is low. If the probability of survival of a single cell during time span t is

$$p = e^{-\lambda t} = E(N_t)/N_0 \quad (2.8)$$

the number of survivors is

$$N_t \sim \text{Binomial}(N_0, E(N_t)/N_0) \quad (2.9)$$

distributed. This can be used as an approximation for the variability in inactivation for any $N_{in} = N_0$ and $N_{out} = N_t$. Note that when N_{in} is large (say $N_{in} > 16\ E(N_{out})/(16-E(N_{out}))$) this distribution is approximated by the normal distribution

$$N_{out} \sim \text{Normal}(E(N_{out}), \sqrt{(E(N_{out})(N_{in}-E(N_{out}))/N_{in})}). \quad (2.10)$$

In contrast to the growth process, inactivation can result in a decrease in the prevalence. Once the number of cells in/on a unit drops to zero, the prevalence will decrease. The probability of this happening in a unit can be derived from the equation above as $(1-E(N_{out})/N_{in})^{Nin}$ which $\approx e^{-E(Nout)}$ when $E(N_{out})/N_{in}$ is small. Hence the predicted prevalence after inactivation, if all units are equal, is

$$P_{out} = P_{in}(1-(1-E(N_{out})/N_{in})^{Nin}). \quad (2.11)$$

2.3.2.3 Partitioning

Partitioning affects the food matrix. It occurs when a major unit is split up into several minor units, as given schematically in Fig 2-3.

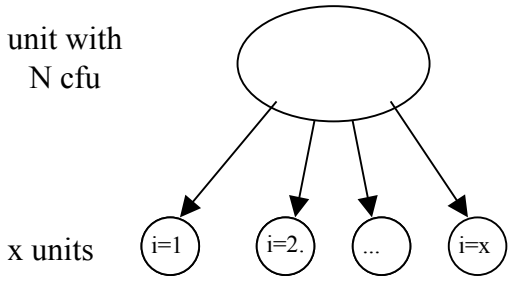

Figure 2-3 Partitioning: A major unit, containing N cfu (particles, spores, cells, etc) is split up in x smaller units i (i= 1..x). The problems to be solved are: (1) what is the number of smaller units with zero cfu and (2) what is the distribution of N_i, the numbers of cfu over the x minor units that are contaminated ?

With the model we have to describe the distribution of the N cells in the major unit over the x smaller units.
First, consider a possible change in prevalence. If, due to sampling effects, any of the smaller units contains zero cells, the prevalence will decrease. Assuming random sampling, the probability of an 'empty' smaller unit, P(zero cells in smaller unit) = P(0) = $(1\text{-}1/x)^N$, so the new prevalence

$$P' = P \times (1-(1-1/x)^N). \tag{2.12}$$

The expected number of empty smaller units is then

$$E(x_0) = x.\mathrm{P}(0) = x^{1-N}(x-1)^N. \tag{2.13}$$

Due to the interdependence between the numbers in the smaller units, we were not able to derive the standard deviation in x_0 analytically. It is smaller than expected from a binomial expectation:

$$\sigma(x_0) < \sqrt{(x\ \mathrm{P}(0)(1-\mathrm{P}(0))} = \sqrt{(x^{1-N}(x-1)^N - x^{1-2N}(x-1)^{2N})} \tag{2.14}$$

If P(0) is small (that if is N is large and x is relatively small), x_0 can be assumed to have a Poisson(E(x_0)) distribution. Then, the number of empty smaller units in a Monte Carlo is a random sample from a Poisson($x^{1-N}(x-1)^N$) distribution.
Next, consider the distribution of the numbers of cfu per smaller unit. Assuming random distribution and equal sized smaller units, sampling leads to a number of cells N_i' as a sample from a Binomial (N, $1/x$) distribution for one smaller unit i. (Hence the expected number of cells is N/x). If the smaller units are not equal sized, and the smaller unit has size m compared to size M of the major unit, $N_i' \sim$ Binomial (N, m/M) for this one smaller unit.

For a series of i equal sized smaller units, there is a problem of dependence between the samples. In that case it can be derived that

$$N_i' \sim \text{Binomial}[N - \sum_{j=1}^{j=i} N_j' \ , 1/(x\text{-i+1})] \qquad (2.15)$$

for a series of i , as in the following list (with $p = i/x$):

i=1: $N_1' = \text{Bin}(N, p)$,
i=2: $N_2' = \text{Bin}(N - N_1', p/(1-p))$,
i=3: $N_3' = \text{Bin}(N - N_1' - N_2', p/(1-2p))$
...
$i=j$: $N_j' = \text{Bin}(N - {}_{i=1}\Sigma^{j-1} N_i', p/(1-(j-1)p))$ (2.16)
...
$i=x$: $N_x' = N - {}_{i=1}\Sigma^{x-1} N_i'$

This list of equations (2.16) can be implemented in a Monte Carlo model, representing the variability distribution of N_i over the smaller units. The whole series (2.16) may be implemented in a spreadsheet, but this may be computationally problematic when x is large. As an approximation, the distribution of N_i can be calculated, if the number of smaller units with zero cfu, x_0, is determined as given above (equations (2.12) to (2.14)). The distribution of N_i over the x-x_0 contaminated smaller units is then assumed to be Binomial (N-x+ x_0, $1/(x\text{-}x_0)$) + 1 cells per unit. Some experimental simulations showed us that this quite good an approximation (*data not shown*).

2.3.2.4 Mixing

Mixing also affects the food matrix and is the opposite of partitioning. In a 'mixing' process units are gathered to form a new unit, as shown in Fig 2-4.

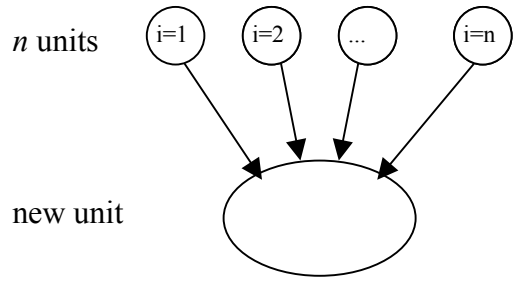

Figure 2-4 Mixing: n units, containing N_i cfu (particles, spores, cells, etc) in all n units i are put together to form a new larger unit. This larger unit will contain $N' = \Sigma_n N_i$ cfu. We want to know the distribution of N', given the distribution of N_i.

When n equal units are mixed to one, after mixing $P' = 1-(1-P)^n$ (assuming random mixing) and $N' = \Sigma_n N$. When differently sized units i are mixed, $P' = 1-\Pi(1-P_i)$, and again $N' = \Sigma_n N_i$.
These equations can be implemented directly into a spreadsheet model, but when n is large, this may be computationally inconvenient or even impossible. In that case you may use the central limit theorem as an approximation.
The central limit theorem states that if $S_n = \Sigma_n X_i$ with X_i independent and with the same mean (μ) and standard deviation (σ) , $(S_n - n\mu)/\sigma\sqrt{n}$ is asymptotically normal.
If we mix several smaller units to a large one, the total number of cells in the larger unit can be regarded as the sum of independent random variables with the same distribution. (But it need not be: There can be dependence (e.g. if the distributions involve uncertainty), and the distributions may be non-identical.)
We can apply the central limit theorem, if we know the mean and standard deviation of this distribution. This is the case if the distribution of the numbers of cells in the n units is a well known statistical distribution, but it need not be the case if the distribution of numbers of cells is 'non-standard'.

1) 'Standard' Distribution
If the distribution is a known distribution (Normal, Uniform, BetaPert etc.) we know mean and standard deviation and we should be able to apply the central limit theorem.

2) Non-standard distribution
If the distribution is 'non standard', that is if it is some function of a known distribution, we may have a problem. For example, if the number of spores on a broccoli trunk is $10^{\text{Uniform(a,b)}}$ distributed, the mean and standard deviation of the number of spores per trunk are not straightforward. In that case we can apply the following theory:
If we have probability density function $f(x)$ and a function $g(u)$ that describes the transformation of the function (e.g. $g(u) = 10^u$ means we take $10^{\text{prob. function } f(x)}$. If $f(x)$ is the normal probability density and $g(u) = e^u$, g(u) is the lognormal probability density.)
Then by definition, for any $f(x)$ and $g(x)$:

$$\int f(x)dx = 1 \tag{2.17}$$

$$\int f(x)xdx = E(x) = \mu_x \tag{2.18}$$

$$\int f(x)g(x)dx = E(g(x)) = \mu_{g(x)} \tag{2.19}$$

$$\int f(x)g(x)^2 dx = \text{var}_{g(x)} + \mu_{g(x)}^{\ 2} \tag{2.20}$$

If you take the appropriate bounds of the integrals and use summation when you are dealing with discrete distributions, this applies for all probability functions and their distributions.

The values of $\mu_{g(x)}$ and $var_{g(x)}$ in equations (2.19) and (2.20) can be applied in the central limit theorem as mean and variance of the normal distribution $N(n\mu,\sigma\sqrt{n})$ for the sum of n samples. For example, the number of spores in a batch containing n broccoli trunks is a random sample from this $N(n\mu,\sigma\sqrt{n})$ distribution.

Unfortunately a special problem arises in application of this method when summing samples from the lognormal distribution, or any other distribution where $g(x)$ is a power function like 10^x. Such a distribution has an extremely long tail. In a Monte Carlo (or Latin Hypercube) sample the probability of sampling from this tail is extremely low. However, the large values in this tail are dominating the size of the (analytic) variance of the distributions: the variance in a sample from a lognormal distribution is much lower than the analytic variance. The result is that the value of $N' = \Sigma_n N_i$ may be too large when it is approximated by sampling from a $N(n\mu,\sigma\sqrt{n})$ distribution, derived by using the central limit theorem and equations (2.17) through (2.20). As illustrated below, it implies that we cannot use this approximation when n is (relatively) small. In that case, simulation is the only option.

For example, consider n broccoli trunks. The distribution of the number of spores per trunk is known to be lognormal, characterised by a probability density function $f(x) = N(2,1)$ and the transformation function $g(u) = 10^u$. This implies that the median number of spores is 10^2, and by calculating equations (2.19) and (2.20) it can be derived that $\mu = 10^{2 + \ln(10)/2} \approx 1417$ and $\sigma = \sqrt{(10^{4 + 2\ln(10)} - 10^{4+\ln(10)})} \approx 20022$. We are now interested in the probability distributions of the number of spores in batches of both n=100 and n=1000 broccoli trunks.

In figures 2-5 and 2-6 the result of 1000 Monte Carlo's (MC's) with n=100 and n= 1000 iterations is compared with the Normal $N(n\mu,\sigma\sqrt{n})$ distribution that the central limit theorem (CLT) predicts. Each MC predicts a random batch. The results of the MC's are sorted and

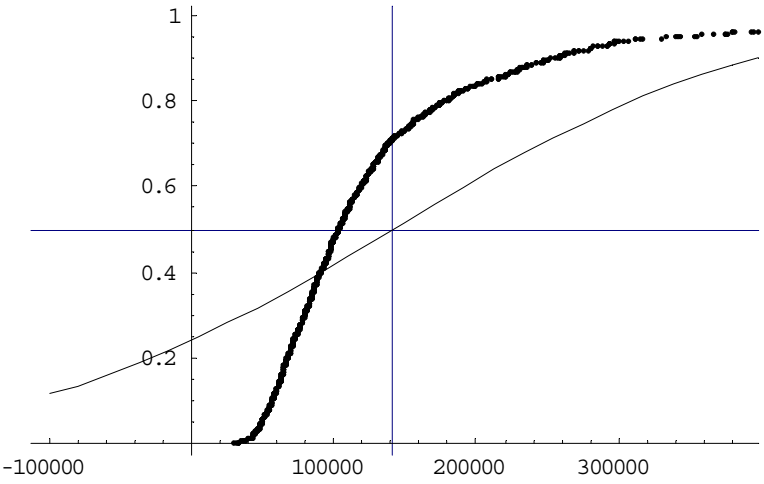

Figure 2-5 Comparison of Monte Carlo results and the prediction using the central limit theorem with n=100. The cumulative distribution is shown with the sum of spores on the x-axis and the probability on the y-axis. For the MC the mean is 13.9×10^4, the median is 10.3×10^4 and the standard deviation is 12.9×10^4. For the CLT method the mean and median are 14.2×10^4, and the standard deviation is 20.0×10^4.

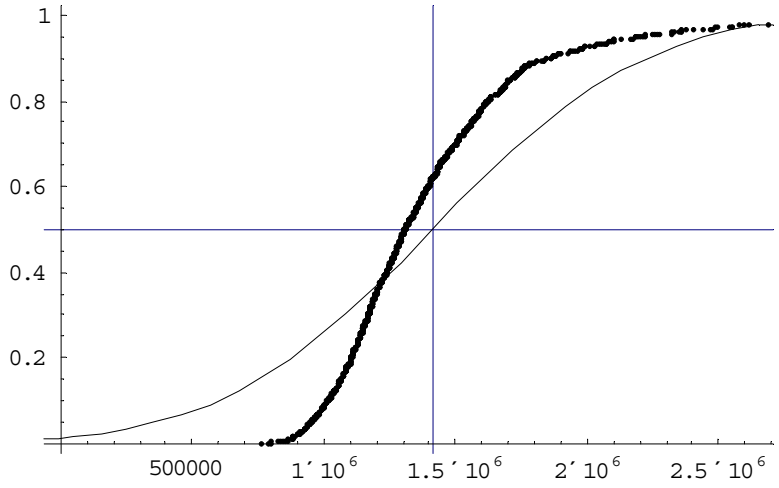

Figure 2-6 Comparison of Monte Carlo results and the prediction using the central limit theorem with n=1000. The cumulative distribution is shown with the sum of spores on the x-axis and the probability on the y-axis. For the MC the mean is 14.1×10^5, the median is 13.1×10^5 and the standard deviation is $47.3\ .10^4$. For the CLT method the mean and median are 14.2×10^5, and the standard deviation is 63.3×10^5.

plotted on a scale from 0 to 1, so that, when the two methods match, the MC should largely overlap the normal distribution predicted by the CLT. The figures illustrate that this is not the case. The median and the standard deviation of the MC-distribution is lower than predicted by applying the CLT. This effect is largest when n is relatively small.
The problem outlined above, that the sum of a (large) number of samples from a lognormal or similar long tailed distribution can not be predicted by a known distribution, may be solved by selecting the Gamma distribution as the initial distribution (for e.g. the number of cfu on the broccoli trunks). When n samples from a Gamma distribution $\Gamma(\alpha,\beta)$ are summed up, this sum is $\Gamma(\alpha n,\beta)$ distributed (Johnson and Kotz 1970), which is easy to implement in a spreadsheet. However, the choice for a Gamma distribution must be realistic: data or expert opinion must be in accordance with the shape of the Gamma distribution. In this context, an important feature of the Gamma distribution is that, if the standard deviation is larger than the mean (as in the example discussed above), the mode of the distribution is at zero. This means that if the initial distribution has a long tail (so the standard deviation is large) and a most likely value larger than zero, the gamma distribution cannot be applied.

2.3.2.5 Removal

Removal is a process where some units (or parts of units) are selected and removed from the production process. Examples are the rejection of carcasses by veterinary inspectors in the slaughter house or the discarding of 'ugly' vegetables. If the removal would be a random process, it would have no effect on the risk assessment. However, the process is usually

performed because there is a presumed relation with microbial contamination: (heavily) contaminated units are discarded more often than lightly or non contaminated units.
A graphical representation is given in Fig 2-7

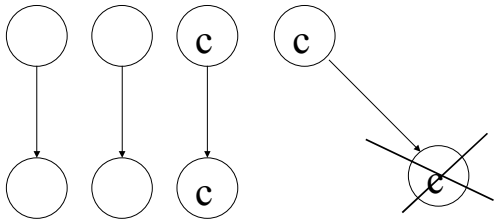

Figure 2-7 In the basic process 'removal' a fraction of the units is removed from the food pathway. Contaminated units (marked 'c') are removed with a higher probability than units that are not contaminated (not marked.) Typically, removal is a selection procedure based on e.g. visible effects of contamination.

Removal is often a subjective process in which the relation between rejection and level of contamination is obscure. Therefore useful mechanistic modelling of the removal process is complex. The simple model presented below is sufficient so far and may be improved in the future.
If it is assumed that heavily contaminated units are not discarded with a higher probability than lightly contaminated units, removal only affects prevalence, and not the variability distribution of the number of cells over the units. In that case removal can be represented by a factor f, such that the prevalence $P`$ after removal is equal to

$$P`=Pf/(1-P+Pf) \quad \text{with} \quad 0 \leq f \leq 1 \qquad (2.21)$$

The rationale behind this equation is shown when (2.21) is rewritten as $P`/P = f(1-P`)/(1-P)$, or by expressing f as $f = (1-p_c)/(1-p_{not\ c})$, with p_c the probability of removal of a contaminated unit and $p_{not\ c}$ the probability of removal of a not contaminated unit. If f=0 all contaminated heads are removed, if f=1 none of them are removed.
This f may be variable and uncertain, derived from experimental results or expert opinion.

2.3.2.6 Cross contamination

Cross contamination is an important process in food safety, but not well-defined. Several types of cross contamination can be considered. Cross contamination can be the direct transmission of cells from one unit to another, e.g. by the (short and incidental) physical contact between two animals, carcasses, vegetables etc. It can also be indirect transmission, for example via the hands or equipment of a food processor. A third type of contamination is the transmission from outside the food production process, the introduction of cells from vermin, dirty towels etc. Strictly speaking, this is not 'cross' contamination. It may be an important process if it refers to the introduction of (substantial quantities of) the hazard

considered into the food chain. If it is believed to be relevant, it should be incorporated in the exposure assessment separately.
Ignoring this last type of cross contamination, these cross contamination processes have in common that the prevalence is increased and that the number of cells remains about equal, and is only somewhat redistributed between the units.
For the prevalence we can then apply the same equation (2.21) as for removal, but now $f>1$ (Cassin et al. 1998a, Fazil, A. and Lammerding, A., *unpublished*):

$$P`=P.f/(1-P+Pf) \quad \text{with} \quad f>1 \qquad (2.22)$$

The larger f, the larger the impact of cross contamination. Like in the case of the removal process, this f may be variable and uncertain, derived from experimental results or expert opinion. Whether the effect of cross contamination on N is important, and how it is to be modelled, depends on the specific situation analysed.

2.3.2.7 Black Box

As stated above, some processes are to be regarded as 'black boxes'. A process like defeathering in a poultry slaughterhouse, for example, holds elements of removal, cross contamination and possibly involves growth or inactivation. If there is no proposal for an alternative scenario including this processing step, and the knowledge about its potential effects on the microbial hazard considered is limited, this might be regarded as 'one processing step'. The transmission is most easily modelled by linear models, that is by assuming that the number of cells changes by an uncertain and possibly variable factor: $N' = N.x$, so

$$\log(N') = \log(N) + x \,, \qquad (2.23)$$

with x a real number), and that the prevalence may change with a factor as modelled in the removal and cross contamination basic processes

$$P`=P.f/(1-P+Pf) \qquad \text{with} \quad f>0. \qquad (2.24)$$

The values of these factors will have to be estimated by data from the process considered or expert opinion. In both cases variability and uncertainty will have to be incorporated explicitly in the model. If there are a series of input-output data available, these may show that the input output relation is not linear. In that case another relation will have to be modelled, if possible with a mechanistic model, including relevant parameters.

3. Exposure assessment of *Bacillus cereus* in broccoli puree

written with F. Carlin, INRA Avignon, France

3.1 Statement of purpose QMRA *B. cereus* in broccoli puree

The aim of the model described and discussed here, is to perform an exposure assessment of *B. cereus* in 380 g broccoli puree packages produced by company X. Endpoint of the exposure assessment is the prevalence of contaminated products and the number of colony forming units in contaminated products at the moment that the consumer takes the puree packages out of its refrigerator for consumption.
We estimate the number of *B. cereus* colony forming units, which are both spores and vegetative cells, in the end product. The difference between these two states of *B. cereus* cells may be highly relevant for the effect of consumer processing after taking the product from the refrigerator and for the effect of *B. cereus* intake. Sporulation along the food pathway is neglected, as it is assumed that this will only occur at the end of the growth phase, which will with high probability be associated with spoilage. To address this point, we assess the frequency in which growth until the maximum population density is reached.
As 'alternative scenario's', we evaluate the effect of keeping to the 'sell by date' that is printed on the broccoli puree packages, and the effect of different temperature profiles in the refrigerators at home of the consumer.

3.2 The food pathway

For broccoli puree part of the food pathway is the industrial process from company X. This process is well controlled and described. However, not all details can be made public. The company was willing to give sufficient details for a quantitative risk assessment. The food pathway after the industrial processing, at retail and at the consumers homes, is less well controlled, it is variable and data are scarce. Therefore assumptions have to be made here. Below, the food pathway is split in two: first the industrial process and next the retail/consumer phase. The moment the product leaves the factory is an important point in the process. At this point industry may check the quality of the product. After this point the industry is no longer in (full) control.
It is assumed that the acidity and the water activity of the product are constant throughout the (relevant part of the) process (pH = 6, a_w= 0.99). The relevant characteristics of the food pathway are a description of the 'unit' (size or weight), and time and temperature of the processing steps.

3.2.1 Industrial process description

The industrial process description has been send to us by company X. We asked for a complete description of the process with specific information on the most likely and minimum and maximum values for the quantities used (i.e. the unit sizes), the processing times and processing temperatures of each step in the process. For overview, we first give an outline of the process. Next it is given in detail.

3.2.1.1 Outline

An outline of the process is given schematically in Fig 3-1 and in Table 3-1 below.

Table 3-1 The industrial process split up in processing steps. For each step the unit size, the processing time and the process temperature are given as mean values. Time values are cumulative.

	process step	unit	time (h)	temp (°C)
1	**start**	broc. heads (400 g each)	0	
2	**storage**	..	196.0	5.7
3	**trimming**	..	196.0	5.7
4	**storage**	..	224.0	7.0
5	**blanching**	280 kg batch	224.2	85.0
6	**storage**	..	224.3	20.0
7	**cooking**	..	225.1	97.5
8	**blending**	..	225.3	70.0
9	**adding ingredients**	325 kg batch	225.8	65.0
10	**vacuum packing**	380 g packs	226.3	58.3
11	**pasteurisation**	..	232.4	70.0
12	**cooling**	..	232.7	drops
13	**storage**	..	284.7	4.0

3.2.1.2 Detailed

The detailed process description has been provided by company X (*data not shown*). For each processing step unit size, processing time and processing temperature are given as minimum, most likely and maximum values, based on data or expert opinion. The final transmission model has been constructed for 280 kg broccoli batches with 45 kg of ingredients, and for 380 g vacuum packed packages of broccoli puree only. Two of the ingredients added at processing step 9, starch (10 kg) and milk powder (5 kg), may contain *B. cereus* spores. The pH of the broccoli trunks is 6.5, after blanching the pH of the product is about 6. The water activity of the broccoli trunks is about $a_w = 0.95$, after blanching it is about 0.99.

For the transmission model, one of the most important processing steps is cooking. A detailed description of the temperature profile of this process, provided by company X, is given in Table 3-2 and in Fig 3-2.

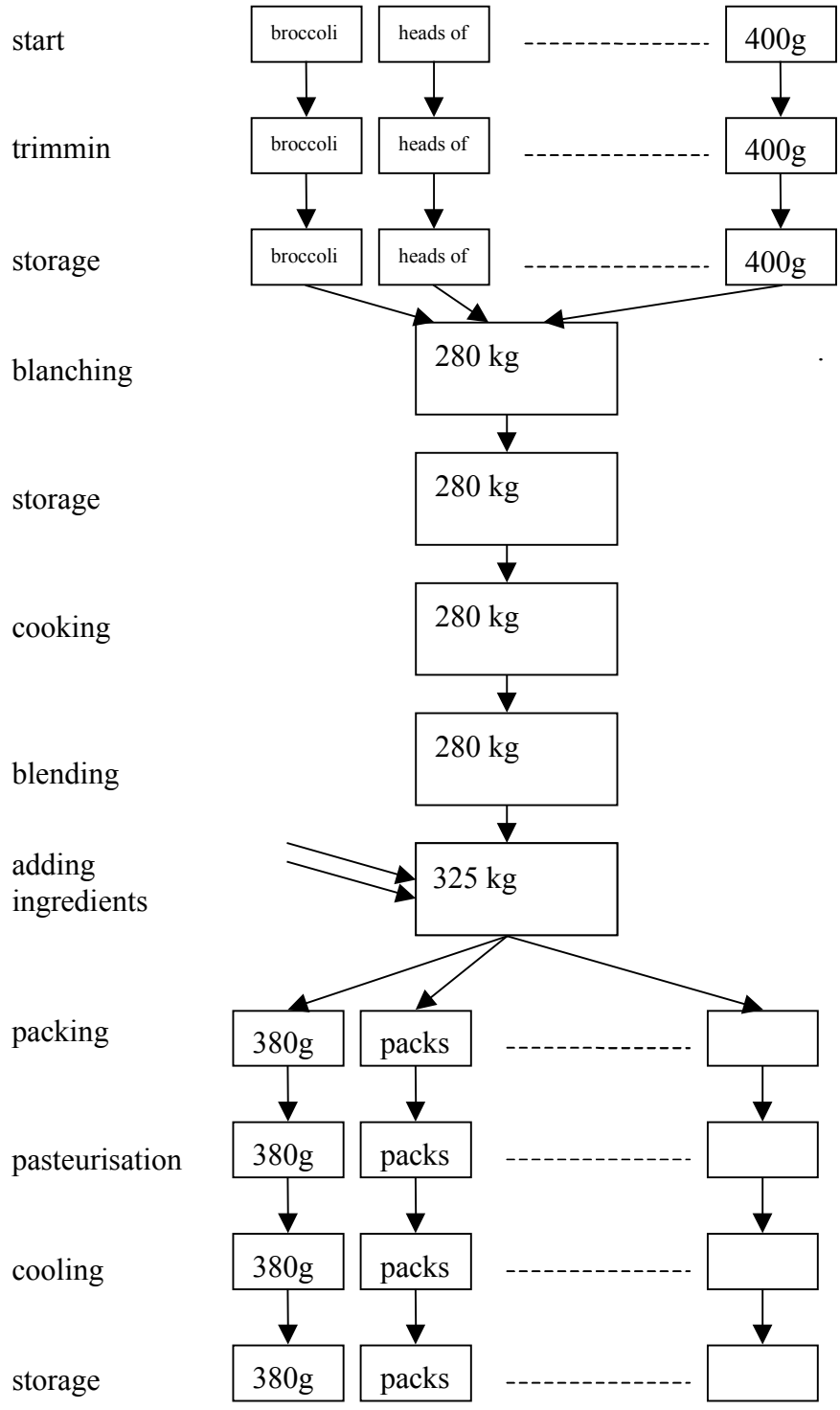

Figure 3-1 A schematic representation of the broccoli puree production process.

Table 3-2 Temperature profile of the cooking process. Temperatures are given at different time steps, both in the oven and at the core of the broccoli. Time t=0 is the start of the blanching process, which is not relevant here and therefore not included.

time (min)	oven (°C)	core broccoli(°C)
39	40.9	32
40	44.8	36.8
45	59.1	57
63	99.2	99.7
78	99.7	100.4
88	99.9	100.4
89	100.2	100.5
90	100.7	100.7

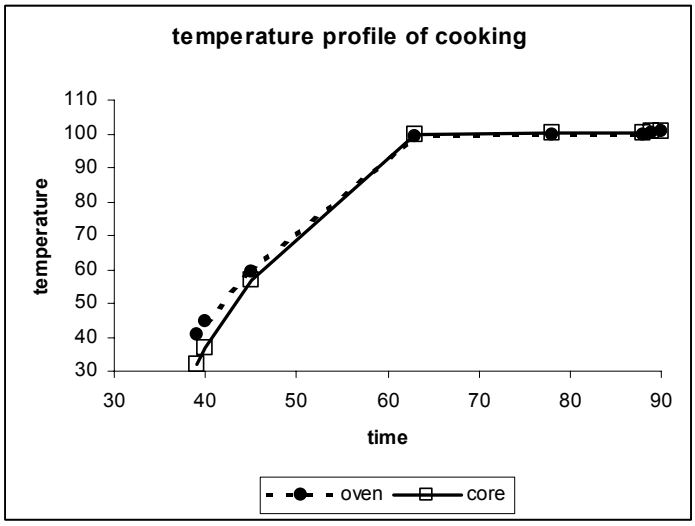

Figure 3-2 The temperature profile given in table 3-2. Data points are joined by straight lines, as in the temperature profile used to calculate the effect of inactivation (see 3.4.2.2.2).

3.2.2 Retailer and consumer handling

To describe the transmission of *B. cereus* in the process from storage at the factory until the moment the consumer takes the product from the fridge, we use information supplied by company X (*unpublished*), and information gathered by SYNAFAP (Litman 2000).
We need information on the probability distributions of times and temperatures of each part of the process. Unit sizes are fixed at 380 g packages all over. For overview, we first give an outline of the process. Next it is given in detail.

3.2.2.1 Outline

The processes from which the time temperature profiles are needed are:
-transport from factory to retail;
-retail (supermarket);
-transport from retail to consumer household;
-storage in the consumer fridge.
Table 3-3 presents the means of the process characteristics.

Table 3-3 The retail/consumer part of the food pathway split up in processing steps. For each step the unit size, the processing time and the process temperature are given in mean values. Time values are cumulative with Table 3-1**.**

	process step	unit	time (h)	temp (°C)
14	**transport**	380 g packs	323.7	4.67
15	**retail**	...	472.2	4
16	**transport**	...	472.9	11.5
17	**home fridge**	...	578.1	6.64

3.2.2.2 Detailed

3.2.2.2.1 Transport from factory to retail

Company X supplied us with the expert opinion that transport takes between 18 (minimum) and 72 hours (maximum, with most likely value 36 hours. Minimum and most likely temperature is 4 °C, with maximum 8°C.

3.2.2.2.2 Retail (supermarket)

At the moment the product leaves the factory, the sell by date (SBD) is set at 21 days later. At retail 80% is sold until 1 week before passing the SBD, and 20% is sold in the last week.
For temperature we have French data (Pierre 1996), with mean 4°C and standard deviation 2.17.

3.2.2.2.3 Transport from retail to consumer household

For transport time we have British data (Evans et al. 1991) and Dutch data (Voedingscentrum, 1999). In the UK transport time from retail until consumer fridge has a mean of 42.8 minutes, with standard deviation 18.7, in the Netherlands time from retail to home has a mean of 7.9 minutes and a standard deviation of 5.9 minutes, after which 95% of the people store the product in the fridge within 5 minutes.
The temperature during transport is largely unknown. It has been found that the temperature in the back of a car may raise up to 40 °C, but that is an extreme value. Evans (1998) has

some information, but this cannot be used in the present form. Expert opinion of Carlin (*unpublished*) states minimum 4°C, most likely 10°C and maximum 25 °C.

3.2.2.2.4 Storage in the consumer refrigerator

For storage time in the fridge it is proposed in consultation with company X that 80% is stored less than one week, 15 % between one and two weeks, 5% until three weeks. 5% of the packs is consumed after the SBD.
The data collected by Litman (2000), allow us to assess home fridge temperatures of the consumer. Table 4 of her report shows observed temperatures in domestic fridges. From this report we constructed Table 3-4 for the distribution of mean temperatures over fridges.

Table 3-4 Reported values of home fridges. Values are the fraction of fridges with reported values higher than the one given in the heading. (Data from Litman 2000)

	>5	**>6**	**>7**	**>8**	**>9**	**>10**	**>11**	**reference**
UK 1	0.71	0.54	0.33	0.19	0.07	0.02	0.01	Evans et al. 1991
France 1		0.7		0.41				AFF, 2000
Greece				0.55		0.25		Sergelidis et al. 1997
NL	0.7		0.3		0.03		0.02	Notermans et al. 1997
UK 2	0.82	0.7	0.56	0.25	0.08	0.04	0.015	Johnson et al. 1998
France 2		0.52				0.18		Anonymus, 1999
France 3	0.8					0.06		Guingois, 2000

The data for UK1 and UK2 are extracted from the original reports(UK1) or the graphs therein(UK2).

3.2.3 Identification of basic processes of the MPRM

Following the modular process risk model (MPRM) framework, the food pathway is split up in basic processes, growth, inactivation, mixing, partitioning, removal, cross contamination or ‘black box’.
In Table 3-5 all processing steps identified above are listed. A basic process is assigned to each of the processing steps. For some steps it was difficult to assign one basic process, for example when mixing occurs at a high temperature which may give rise to inactivation. In that case two basic processes are assigned to a processing step. In the MPRM these processes can be modelled independently.
As will be explained below, after a first analysis of the model, it appeared that the food pathway until the adding of the ingredients (step 9) can be neglected in the exposure assessment, as most spores are killed during the cooking processing step. Hence, the final model consists of a list of basic models as given in the right column of Table 3-2.

Table 3-5 Basic models that describe the transmission of *B. cereus* along the food pathway.

	processing step	basic process	implemented basic process
1	start		
2	storage	growth	
3	trimming	removal	
4	storage	growth	
5	mixing & blanching	mixing, inactivation	
6	storage	growth	
7	cooking	inactivation	
8	blending	inactivation	
9	adding ingredients	mixing, inactivation	START
10	vacuum packing	partitioning	partitioning
11	pasteurisation	inactivation	inactivation
12	cooling	inactivation	inactivation
		growth	growth
13	storage	growth	growth
14	transport	growth	growth
15	retail	growth	growth
16	transport	growth	growth
17	home fridge	growth	growth

3.3 Microbiological data

Microbiological data are data on *B. cereus* relevant for the exposure assessment.

3.3.1 Raw material and initial contamination

The initial contamination data given in Table 3-6 are based on unpublished data and expert opinion of Frederic Carlin.

Table 3-6 Initial contamination data: minimum, most likely and maximum value of the concentration of *B. cereus* on different products.

Product	Minimum	Most likely value	Maximum
Broccoli	10^{-1} cfu/g (1 spore in 10 g)	1 cfu/g	10^{2} cfu/g
Milk powder	10^{-1} cfu/g	10 cfu/g	10^{4} cfu/g
Starch	10^{-1} cfu/g	10 cfu/g	10^{3} cfu/g

From INRA and company X we have information that the most common types of *B. cereus* found in the ingredients are not psychrotrophs. It is therefore assumed that the values for the concentrations in the ingredients in the table above are two log units lower: minimum 10^{-3}, most likely 10^{-1} and maximum10^{1} (starch) or 10^{2} cfu/g (milk powder).

3.3.2 Inactivation

Although more advanced models are being developed, we use a simple log linear Bigelow (D,z) model in this study. The D_T value is the time needed to achieve one log reduction in number of cfu at temperature *T*. The *z* value is the number of degrees C needed to change the *D* value by one log unit.
Inactivation data are gathered in the RASP project by Martinez *et al.* (1999 and 2000). The available *D* and *z* values as used in this study are listed in the table below.

Table 3-7 D_{90} and z values of four different *B. cereus* strains, as used in the exposure assessment.

	D_{90}	z	source
avtz415	2.47	7.78	Martinez et al 2000
	(sd log D:0.0225)		
z4222	20.4	13.3	Martinez et al 1999
avz421	39 (sd 1.5)	8.4 (sd 0.1)	Fernandez et al. 1999
l2104	39.4	7.55	Martinez et al 1999

Carlin (*unpublished*) constructed a distribution of *D* values of *B. cereus* as a function of heating temperature from literature data (Gaillard et al. 1998b, Gaillard et al. 1998a, Shehata and Collins 1972, Parry and Gilbert 1980, Couvert et al. 1999, Gonzalez et al. 1997, Mazas et al. 1995, Johnson et al. 1982, Faille et al. 1997, Bradshaw et al. 1975, Fernández et al. 1999, Dufrenne et al. 1994, Dufrenne et al. 1995,Choma et al. 2000 , Wong et al. 1988, Rajkowski and Mikolajcik 1987, Gilbert et al. 1974, ICMSF 1996, Bergère and Cerf 19--.) This is given in Fig 3-3. It summarises differences in strains, heating menstrua, methods and may include some *D* values of the tailing of the survival curve (when given). When there were plenty of data, only the minimum and the maximum are shown. Also, he only data at pH higher than

5.5, in water, phosphate buffer or high water activity heating menstrua are used. Experiments in for instance milk, rice broth, oil, etc are removed.

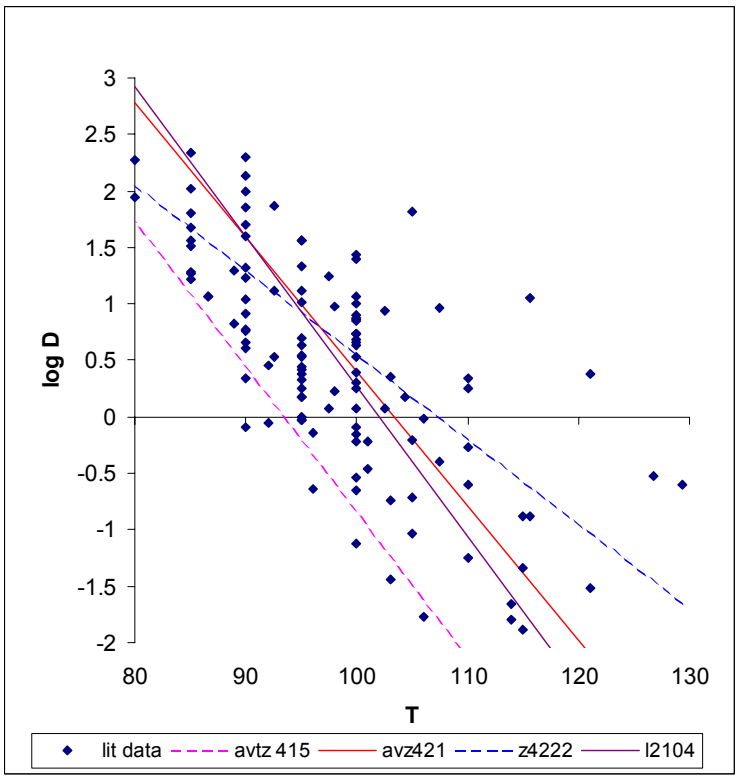

Figure 3-3 *D* values of *B. cereus* in aqueous suspensions at different temperatures. The lines represent the predictions of the models for the four different strains, the dots are data from different literature sources as collected by Carlin (*unpublished*). The graph shows that the selected strains cover the literature data quite well.

3.3.3 Growth

Data on bacterial growth of *Bacillus cereus* are available form within the RASP project from P. Fernandez (*unpublished*) and F. Carlin(*unpublished*).
The data of Fernandez (Table 3-8) are the means of duplicate experiments in nutrient broth, with maximum specific growth and lag time fitted with the Baranyi model (Baranyi et al. 1993) for four *B. cereus* strains. Carlin's data (Table 3-9) are obtained with strain z4222 in broccoli broth. Maximum specific growth rate μ, lag time λ and the maximum population density are fitted with the Baranyi model.

Table 3-8 Calculated parameters of growth curves obtained with *B. cereus* strains in nutrient broth, using the model of Baranyi et al. (1993) over the temperature range 5-30°C. Data shown are the average of two replicate growth curves. (Adopted from Fernandez 2000)

Strain	Incubation Temperature (°C)	Lag time (h)	μ_{max} (h^{-1})	R^2
CIP5127	5	No growth	-	-
	8	No growth	-	-
	10	No growth	-	-
	12	52.65	0.016	0.987
	16	18.00	0.138	0.986
	25	4.74	0.665	0.982
	30	2.33	0.876	0.981
L2104	5	No growth	-	-
	8	No growth	-	-
	10	No growth	-	-
	12	39.73	0.049	0.991
	16	13.11	0.130	0.993
	25	4.47	0.574	0.988
	30	2.0	0.754	0.981
AVZ421	5	No growth	-	-
	8	No growth	-	-
	10	No growth	-	-
	12	88.01	0.017	0.991
	16	13.64	0.130	0.993
	25	3.38	0.640	0.974
	30	1.93	1.089	0.969
AVTZ415	5	148.77	0.027	0.991
	8	43.60	0.055	0.998
	10	38.96	0.073	0.997
	12	29.38	0.107	0.996
	16	14.34	0.133	0.977
	25	3.44	0.529	0.973
	30	1.96	0.687	0.991

Table 3-9 Calculated parameters of growth curves obtained with *B. cereus* strain z4222, fitted to the Baranyi model. *se: "Standard error of fitting (estimated standard deviation of the observed independent values, assumed to be constant)" (Adopted from F. Carlin, *unpublished)*.

Strain	Temperature	Lag time(h) (se*)	μ_{max} (h^{-1}) (se)	Maximal population (log cfu/g) (se)
Z4222	20°C	<12	0.167 (0.022)	7.6 (0.27)
	15°C	19 (1.7)	0.106 (0.0067)	7.5 (0.07)
	10°C	52.6 (9.8)	0.056 (0.092)	7.1 (0.2)
	7°C	207.7 (10.65)	0.0219 (0.0018)	6.7 (0.09)

3.3.4 Sporulation & Germination

It is assumed that sporulation occurs at the end of a growth process only. As agreed by the experts within the RASP project it is neglected in the model.
Germination is important when the colony forming units at the end of the exposure assessment are split up into vegetative cells and spores. When a growth process is considered, it starts with germination of (a part of) the spores (the spore lag), followed by a lag phase of the germinated cell (the cell lag). After that, the growth phase starts with cell division of germinated cells.
R. Moezelaar (*pers comm.*) gave as his expert opinion that the spore lag is zero: the lag phase λ that appears in the growth model is totally a cell lag, spore lag can be neglected. The fraction of spores that does not germinate is set as minimum 0, most likely 0.01 and maximum 30 %.

3.3.5 Spoilage

Spoilage may be an important complicating factor in microbial risk assessment. We have some data on spoilage of vegetable products.
Company X studied its own product (*unpublished data*): From tests done at the factory, after 28 days at 5-6°C, the only one visible alteration is some exudate in the product. No off-odours. No pack swelling. All products may be considered as edible.
Carlin et al. 2000b) studied courgette puree, a similar product as broccoli puree. The results are given in Table 3-10.

Table 3-10 Spoilage data on courgette puree, as manufactured by company X. The times to different types of spoilage of the product are noted for different temperatures.

Temperature (°C)	Time to noticeable off-odours (days)	Time to first exudate (days)	Time to pack swelling (days)
20°C	4	no	6
15.2°C	5	no	11
9.8°C	12	20	40
6.5°C	28	26	> 112
4°C	36	35	> 112

Additionally, Carlin et al. (2000b) found in two replicate experiments on broccoli and potato purée that pack swelling occurs after 5-6 days at 20-25°C for broccoli purée and 12-15 days for potato purée. The products are unacceptable at that time. Exudation and off-odours occur previously, but it is not observed when. At 10°C on broccoli purée, there was no spoilage observed after 32 days; products were considered as acceptable. At 4°C on broccoli purée, there was no spoilage observed after 20 days, and and there was unclear acceptability after 46 days, without off-odours or pack swelling.

3.4 The exposure assessment model

The exposure assessment model describes the transmission of the hazard, *Bacillus cereus*, through the food pathway. Data from the food pathway and the microorganism have to be integrated in one structure. This can be done at several levels of detail. The appropriate level is one that is best suited to answer the research question and which best compromises between completeness and simplicity. As above, the food pathway is split up in two, the industrial processing and the retail/consumer phase.

3.4.1 Modelling the Food pathway

Below, we start with a full model of the industrial processing. Next, the industrial process is simplified by neglecting those processing steps that have no relevant impact on the transmission of the hazard. The modelling of partitioning of the batch over packages, an important non microbiological processing step, is explained.

3.4.1.1 Full model until the end of industrial processing

The first model describes the whole process of company X. As indicated in Table 3-11, processing times and temperatures are implemented as BetaPert distribution, that represents variability per iteration, that is per batch. The minimum, most likely and maximum values used are those given by the experts. The values given for the cooking temperature are derived

from the temperature profile as explained below (3.4.2.2.2.). The cooling process, in which the temperature drops form about 80 to about 4 °C, is split in two, because the temperatures are first in an 'inactivation' rang, and later in a 'growth' range. The temperature distributions given for the cooling process are derived from the temperature profile that were available from company X. They were treated similarly as the profiles of the cooking process (see 3.2.1.2. and 3.4.2.2.2). The results of this exercise are unpublished as the cooling process appears to be of minor importance in the total analysis.
Storage at temperatures which may allow growth, is neglected until the last two processing steps. This has two reasons. At first there is very little (negligible) growth and secondly it should be realised that 'growth' is the increase in numbers of vegetative cells, which are inactivated at high rates in the inactivation steps. The inactivation models used concern spores, and it is improbable that sporulation will occur in the industrial process. Therefore the model is a 'spore'-model until the last two industrial processing steps.

Table 3-11. The industrial processing steps and the attending distributions used to describe time and temperature of these processing steps. The BetaPert distributions depend on the minimum, most likely and maximum values as derived from the processing description provided, and describe per batch variability.

[1] From the temperature profile provided, it has been derived that 40% of the time the temperature is in the 'inactivation range', and 60% in the 'growth range', with equivalent temperatures given.

	processing step	**t (h)**	**T (°C)**
1	start		
2	storage		
3	trimming		
4	storage		
5	mixing & blanching	BPert(0,1/4,1.0001/4)	BPert(80,85,90)
6	storage		
7	cooking	BPert(4/6,45/60,5/6)	BPert(93,98,100)
8	blending	BPert(5/60,1/6,15/60)	BPert(60,70,80)
9	adding ingredients	BPert(25/60,35/60,40/60)	BPert(50,65,70)
10	vacuum packing	BPert(25/60,30/60,45/60)	BPert(50,57.5,70)
11	pasteurisation	BPert(250/60,350/60,400/60)	70
12	cooling	BPert(0.75,5/6,1)	BPert(69,71,73)[1] BPert(12,14,17)[1]
13	storage	BPert(0,36,7*24)	BPert(3,4,5)

3.4.1.2 Simplified model until end of industrial processing

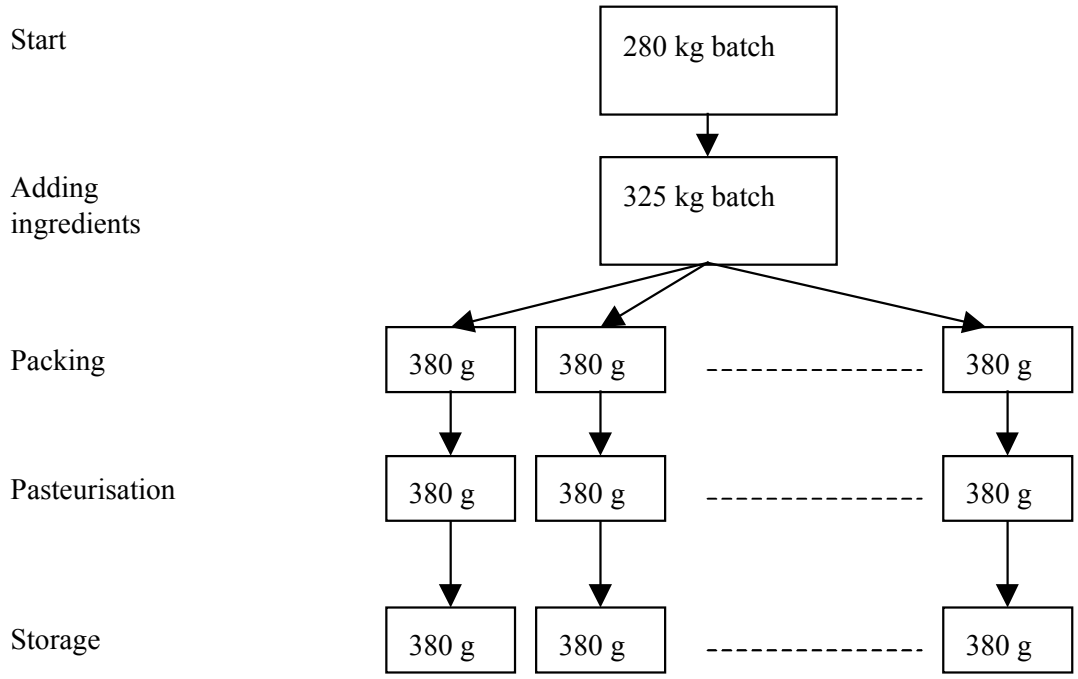

Figure 3-4 The simplified model for industrial processing starts with batches of cooked broccoli puree and ends with packages leaving the factory.

From the first analysis it appears that cooking at step 7 eliminated almost all *B. cereus* spores on the broccoli. The ingredients starch and milk protein that are added at step 9, however, are contaminated to a significant level. Therefore, in a simplified model, we start the process at the moment of adding the ingredients in the batch. Processes with a minor effect on the cells are neglected. The process then looks as given in Fig 3-5:

3.4.1.2.1 Partitioning

Along this pathway the only non-microbiological process is the partitioning process during packing. The mathematics of partitioning are described in paragraph 2.3.2.3.
It is assumed that in this process the spores in the 325 kg batch are randomly distributed over x = 325000/380 = 855 packages of 380 g. By change, some packages may not contain any *B. cereus* spores. Such packages will never constitute a risk With N the number of spores in the batch before packaging, equation (2.12) shows that the probability that a package will not contain any *B. cereus* is $P(0) = 0.99883^N$.
When x is as large as 855, the partitioning process cannot be simulated exactly. As an approximation, the distribution of the numbers of spores N_i over the x-x_0 contaminated packages is assumed to be Poisson($(N-x+x_0)/(x-x_0)$) + 1 cells per package. Some simulation trials showed us that this quite good an approximation for the partitioning process in the current situation (*data not shown*).

3.4.1.3 The model from the end of industrial processing until the consumer fridge

At the retail/consumer phase, time and temperature distributions are derived from the data.

3.4.1.3.1 Transport from factory to retail

For transport from factory to retail the minimum, most likely and maximum values for time and temperature are implemented in BetaPert distributions. The most likely value for the temperature is set a little higher than the minimum (4.01°C) for mathematical reasons.

3.4.1.3.2 Retail (supermarket)

At retail the distribution of temperature is set at Normal N(4,2.17). (Pierre 1996). The time distribution is more complicated. The commercial department of company X proposed that in shops, 80 % are sold within 14 days and 20 % between 14 days and 21 days. (All supermarkets remove products with exceeded sell-by-dates after 21 days.) It is suggested to take a homogeneous distribution during these periods ([80/14]% of products stay one day, [80/14]% stay 2 days, etc…[80/14]% stay 14 days, [20/7]% stay 15 days, …[20/7]% stay 21 days). This suggestion is implemented in the model as 80% sold until seven days before the sell by date (SBD), the rest sold in the last week before SBD, both with a uniform distribution.

From this stage on it is assumed that the distributions describe 'per package' variability and no longer 'per batch' variability. This means that it is assumed that packages originating from one batch experience the same conditions until retail, and are independent after that. This may not be realistic, but it is considered to be the best compromise between computational simplicity and reality.

3.4.1.3.3 Transport from retail to consumer household

For transport from retail to consumer, time is assumed to be Gamma distributed. The Gamma distribution is chosen (and not for example a normal distribution), because it is generally applied to describe (complex) waiting time distributions. Also, the Gamma distribution is always positive and has a longer tail than the normal distribution. The Gamma distribution describing the UK data (mean 42.8, S.D. 18.7) is shown in Fig 3-5., where it is compared to the normal distribution with the same characteristics.

The Dutch data differ significantly from the British data. This is illustrated for the time between retail and home in Fig 3-6.

We use the Gamma(5.24, 8.17) distribution fitted through the UK data in the exposure assessment, as UK travelling distances will probably closer top the European mean than in a densely populated country like the Netherlands.

It is assumed that the distribution of temperatures during transport is BetaPert with the estimated parameters

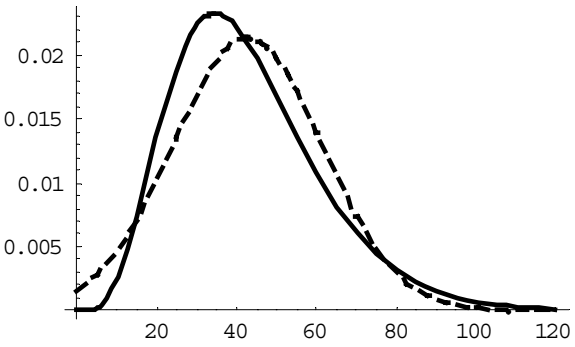

Figure 3-5 Comparison of the Gamma distribution (solid line) and the normal distribution (dashed line) with the same mean and standard deviation, for the UK transport times from retail to home. The Gamma distribution is selected because it has no negative value and has a longer tail.

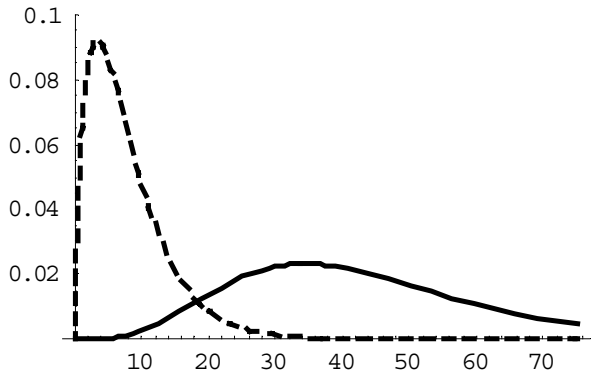

Figure 3-6 Comparison of the Gamma distributions fitted through the Dutch data (dashed line) and the British data (solid line) on transport times from retail to home.

3.4.1.3.4 Storage in the consumer refrigerator

Where the distribution of storage times at the consumers homes is concerned, the proposed distribution (80% consumed within a week, 15 % within two weeks and 5% within three weeks) is not consistent with the assumption that 95% of the consumers respect the SBD. As the latter assumption is considered less uncertain than the distribution of times proposed, the distribution of storage times has been adapted as given below.

First, to describe the distribution of times that the products are kept in the fridges, we choose an exponential distribution. This distribution is characterised by one parameter only (see 2.3.1.1.6.) and is a 'natural' choice because it describes the waiting time between two events. Next, it is assumed that consumer behavior is influenced by the SBD, such that the storage time depends on the sell by date on the pack. If PD is the day of purchase, SBD-PD is the time between purchase and sell by date, that is the consumer storage time until the SBD is reached. Knowing that the 95% quantile of the exponential distribution lies at 3 × its mean, the exponential distribution which gives 5% probability of a storage time exceeding the SBD

has a mean μ = (SBD-PD)/3. Here, PD is the 'zero point' of the distribution: a consumer won't eat a product before she buys it. As PD is variable, μ is variable too

The resulting distributions for the storage time in the consumer fridge and the days past the SBD that the product is taken from the fridge, are shown in Fig 3-7. These distributions are the result of 10000 iterations of a Monte Carlo simulation (actually a LHS) of the process (from the moment the product leaves the factory until the end of consumer storage) in @Risk. The distribution of storage times in the consumer fridge has a mean 3.73 days, a sd 4.36 days, and a median of 2.23 days. About 30% is eaten within one day of purchase, 83.7% within a week and 96.3% within two weeks. This is comparable to proposed distribution, with values 80% and 95% for these numbers. Of 10000 packages, the maximum storage time in the fridge is 43.3 days, consumption more than 26 days past SBD. The mean date of consumption lies 7.5 days before SBD, 5.2% of the packs is consumed after the SBD.

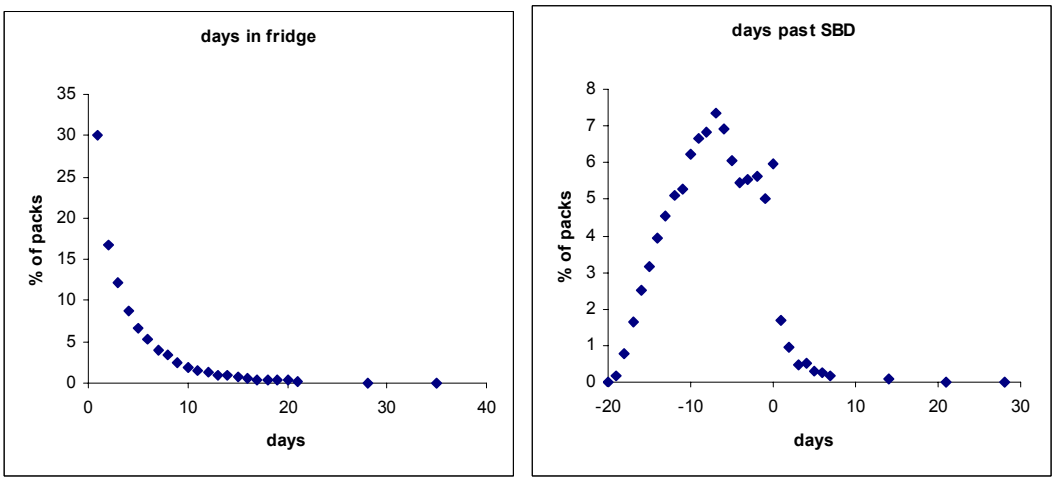

Figure 3-7 Storage times at the consumer fridge, resulting from Monte Carlo simulation. The time the packages are kept in the fridge is assumed to be exponentially distributed, with a variable mean depending on day of purchase (left). Together with information on package handling before consumer storage , this leads to the graph at the right, indicating the number of days past the sell by date (SBD). As it is assumed that the consumer accounts for the SBD, an increased number of packages is consumed shortly before the SBD. 5% of the packages is consumed after the SBD.

Several distributions of temperatures in domestic fridges are derived from the data in Table 3-4. When all these data points are fitted to the Normal distribution with the best fitting mean and standard deviation, the best fitting is N(6.64,2.35). As shown in Fig 3-8 the data show some geographical effects: In northern countries temperatures are roughly lower than in southern countries. We therefore fitted the Greek data and the Dutch data to a normal distribution too. For Greece (two data points only!) this resulted in N(8.32, 2.49) and for the Netherlands in N(5.99,1.83).

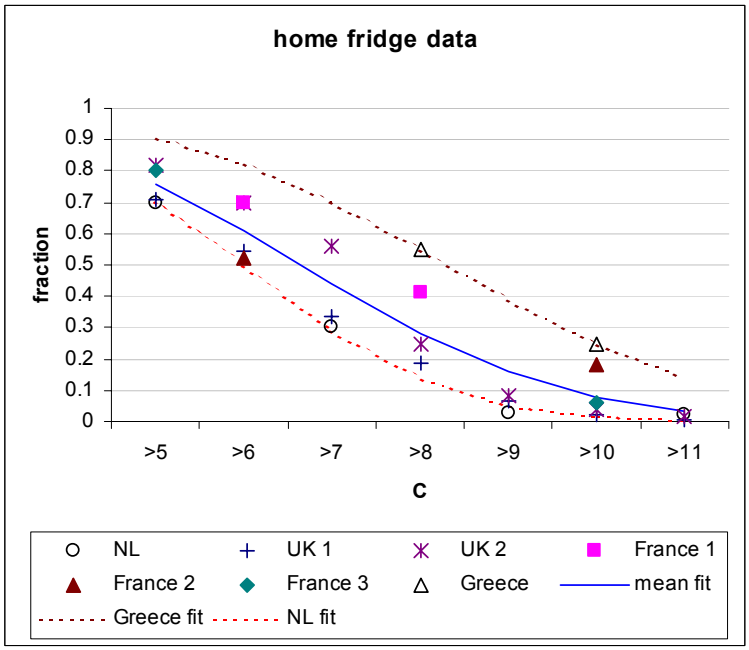

Figure 3-8 The distribution of temperatures in domestic fridges in different European countries. The data from Table 3.4 are plotted and fitted with normal distributions. The solid line shows the fit through the mean of all data points. The dashed lines show the fit through the Greek data and the Dutch data.

In the exposure assessment we use five different 'mean domestic fridge temperature' distributions. The first three are those derived above, based on domestic fridge data:
'Europe' N(6.64,2.35);'North' N(5.99,1.83);'South' N(8.32,2.49).
These distributions represent the mean fridge temperatures over time. Within fridge variability (over time) is again characterised by a normal distribution, with a mean from the distribution above and a sd 1.29 as derived from the data of Evans et al. (1991) (see Appendix 1).
Additionally we consider two 'legislative' distributions. According to the 'international standard' fridges must give storage temperatures between 0 and 10 °C, and the mean at three points should be less than or equal to 5°C. This is interpreted as a Normal (5, 1.89) distribution, where the sd 1.89 is the sd from the BetaPert(0,5,10). This is a 'within fridge' variability, the same for each fridge. (We use a Normal distribution and not a BetaPert because, in this case, this is easier to implement in the spread sheet). The last alternative is a constant 7 °C. This is not realistic, but generally considered to be 'safe'.
The resulting alternative domestic fridge temperature distributions are given in Table 3-12. All this summarises to the assumptions on time and temperature distributions it the trajectory from factory until consumer given in Table 3-13.

Table 3-12 The alternative temperature profiles of domestic fridges. *m* is the mean temperature within a fridge, sampled from the distribution given. *T* the temperature at any moment from a fridge with that mean.

profile	mean fridges	overall
'Europe'	m ~ N(6.64,2.35)	T ~ N(m,1.29)
'North'	m ~ N(5.99,1.83)	T ~ N(m,1.29)
'South'	m ~ N(8.32,2.49)	T ~ N(m,1.29)
'Standard'		T ~ N(5,1.89)
'Fixed'		T = 7°C

Table 3-13 The distributions of temperature and time of the consecutive phases in the retail/consumer phase. For factory and transport variability is per batch, for the other phases it is per package of broccoli. SBD = sell by date = 21 days after leaving the factory; PD = date of purchase.

	temperature (°C)	time	
factory	BetaPert(3,4,5)	BetaPert(0, 1.5,7) days	
transport 1	BetaPert(4,4.01,8)	BetaPert(18,36,72) h	
supermarket	Normal(4,2.17)	80 %	20 %
		until 7 days before SBD	7 days before until SBD
transport 2	BetaPert(4,10,25)	Gamma(5.24,8.17) min	
consumer fridge	'Europe' N(6.64,2.35)	5% past SBD, Exponential ((SBD-PD)/3)	

3.4.2 Microbiology models and the implementation of microbiological data

3.4.2.1 Initial contamination

The values mentioned in Table 3-6 are implemented with a BetaPert distribution as variability per broccoli head and per 5 kg and 10 kg batch of milk powder and starch.
Based on expert opinion, the prevalence is assumed to be 100% everywhere.
Other ingredients are assumed to be not contaminated.

3.4.2.2 Inactivation

3.4.2.2.1 General considerations

Estimates of *D* and *z* values are usually based on inactivation at fixed temperatures (isothermal inactivation). In food production processes temperatures are however not fixed , but may increase and decrease. Estimation of the effects of inactivation is therefore not straightforward.

There are several non-isothermal inactivation processes in the production process. The most important ones are the cooking of the broccoli heads and the pasteurisation of the puree packages. The effect of pasteurisation in terms of time-temperature is given by company X as equivalent times at 70 °C. The cooking process is interpreted as explained in the paragraph below.

3.4.2.2.2 Extracting information from the temperature profile

The temperature profile given in 2.1.2. is a characteristic example of what might happen during cooking in industrial processing. To predict inactivation of spores during this process, we have to construct a model for non-isothermal inactivation. Traditionally, simple inactivation models provide *D* and *z* values for isothermal conditions (that is with a constant temperature). We used the following approach to predict the effect of a non-isothermal process, using the *D*,*z* (Bigelow) model for isothermal inactivation. The aim is to define the isothermal process that gives the same level of inactivation, i.e. to find the equivalent 'isothermal' temperature of the process.

In isothermal conditions, inactivation at temperature *T* is described by the decimal reduction time, the D_T-value, given the *z*-value and the D_r value, the decimal reduction time at $T=T_r$

$$\log(\frac{D_T}{D_r}) = \frac{T_r - T}{z} \qquad (3.1)$$

with by definition

$$d(\log N)/dt = -1/D_T \qquad (3.2)$$

so

$$\log(N_t) = \log(N_0) - t/D_T \qquad (3.3)$$

If the temperature changes with time and is described by *T(t)*, the inactivation can be estimated by summing up or integrating the inactivation over small time intervals *dt*. Taking the integrals for variable *T(t)*, equation (3.2) leads to

$$\log(N_\tau) = \log(N_0) - \int_0^\tau 1/D_{T(t)}\, dt \qquad (3.4)$$

with τ the length of the total time interval considered. Here we assume that with *T* changing over time equation (2) still holds. An important issue is that, by the lack of a good alternative, we assume that the D-value at a certain temperature is the same under isothermal and non-isothermal conditions. Unfortunately there are strong indications that it is not (Fernandez A. et al. 2000)
Next, assume that temperature increases linearly between each two time steps, *i* and *i*+*t*, (with *t* the length of the time interval between the two time steps) so that

$$T(i+t) = a\,t + T(i), \tag{3.5}$$

with $0<t<\tau$ and a constant *a*, so

$$(T(i+t)-T(i))/\,t = a \tag{3.6}$$

By incorporating (3.5) in (3.1) taking the integral of $1/D_{T(t)}$ as in equation (3.4), it can be derived that for each step *i* (see Hayakawa et al. 1969 and Fernandez A. et al. 2000),

$$SC_i = \log\left(\frac{N_{t+i}}{N_i}\right) = \frac{z.10^{\frac{T(i)-T_r}{z}}}{\ln(10)\,D_r} \times \frac{(10^{\frac{T(i+t)-T(i)}{z}}-1)}{T(i+t)-T(i)} \times t \tag{3.7}$$

As we can read the values of $T(i)$, $T(i+t)$ and τ from Table 3-14, and we have values of D_{90} and *z* available, we can calculate the step characteristic $SC_i = \log(N_{t+i}/N_i)$ for each step *i* (definition SC adopted from Van Gerwen and Zwietering 1998):

Table 3-14 Time temperature profile of the cooking process, and the values of the step characteristic SC, indicating the log decrease of the concentration according to equation (3.7).

time	oven	core broccoli	SC_i oven	SC_i core
39	40.9	32	0.00	0.00
40	44.8	36.8	0.00	0.00
45	59.1	57	9.35	10.18
63	99.2	99.7	99.64	119.11
78	99.7	100.4	73.62	87.92
88	99.9	100.4	7.93	8.92
89	100.2	100.5	8.93	9.33
90	100.7	100.7		

For the whole cooking process the sum of all SC_i's is the log decrease of *N*. For example, for strain avtz415, with D_{90} = 2.47 and z = 7.78 (see Table 3-15), Σ SC_i = 199.5 for the oven and Σ SC_i = 235.5 for the core.

These values can be used to calculate equivalent *D* value, that is the mean decimal reduction time for this non isothermal process

$$D_{eq} = \tau / \Sigma SC_i \quad (3.8)$$

and the equivalent constant temperature (for the same time interval τ) at which the same level of inactivation will be reached (in theory, equation (3.1)) is

$$T_{eq} = T_r - z \log(D_{eq}/D_r) \quad (3.9)$$

When equation (3.7) is inserted in this equation, the resulting equation looks horrible. However, it is important to note that D_r, T_r, and τ are cancelled out. The value of the equivalent temperature T_{eq} therefore depends on the *z*-value and the temperature profile only.
For the given temperature profiles and the z-value considered, this results in T_{eq} =97.7 °C and T_{eq} =98.2 °C for the oven and the core of the product respectively. For other z-values in the range of those found for different *B. cereus* strains (between z = 7 and z = 14), T_{eq} may vary between 97.8 and 96.3. With other temperature profiles that were by company X, we got similar results (*data not shown*).
In the risk assessment, for cooking temperature we applied a rather broad range around these values, [min, most likely, max] of [93,98,100] with time [40,45,50] min, (the latter as indicated by company X). We choose a broad temperature range because the derivation outlined here has not been validated, and the variability of the temperature profiles is not known.

3.4.2.3 Growth

3.4.2.3.1 Selection of a primary and a secondary growth model

To model the increase in population size as a function of time and temperature, we have to apply primary and secondary growth models.
In a primary model, the growth curve is given as a function of time. The best option seems to be the Baranyi model (Baranyi et al. 1993), as the available growth data were fitted with this model. However, we select the (simpler) lag exponential model

$$\ln(N_t) = \ln(N_o) + \mu(t-\lambda) \quad (3.10)$$

with N_t the population size at time t, N_o the population size at t=0, μ the specific growth rate (h^{-1}) and λ the lag phase duration (h).
This selection is made for the following reasons:
1) The Baranyi model has a rather complicated structure, and is therefore not easy to implement in a spreadsheet model. The lag-exponential model is simple, its parameters are

readily interpreted as specific growth rate and lag time, and it is easy to implement in a spreadsheet.
2) There must be some 'hidden' assumptions made in the procedure of fitting growth data to the Baranyi model, as the Baranyi model has more parameters than those provided. The values of these parameters could not be recovered.
3) The variability between strains and the variance of the estimates seems much larger than the difference between the Baranyi model and the lag-exponential model. This is illustrated in Fig 3-9. Only in the time period around the end of the lag the difference may be important, but this is neglected.

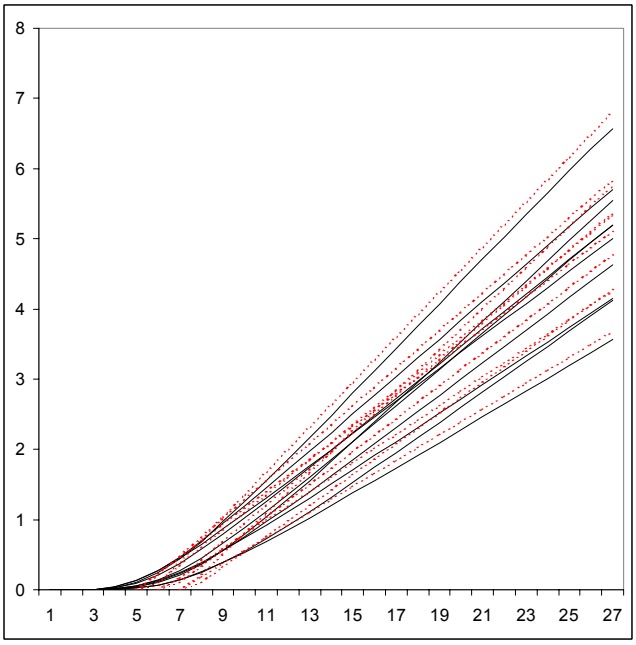

Figure 3-9 The log of the population density N_t plotted versus time, according to an @Risk(Monte Carlo) simulation of the Baranyi model (straight lines) and the lag exponential model (dashed lines), where μ, λ and N_{max} have a Normal distribution derived from the data from Carlin, strain z4222, at 10°C. The dashed lines and the straight lines all fall in the same range. The uncertainty in growth parameter estimates (represented by the different lines) has much more impact than the difference between the two models (the difference between the dashed lines and the straight lines).

Next, we have to choose a second order growth model for the effect of temperature.
As an easy and well established alternative (Ratkowsky et al. 1982, Zwietering et al. 1996), we choose the square root model for temperature and growth rate

$$\mu = b\,(T - T_{min,\mu})^2 \qquad (3.11)$$

in which $T_{min,\mu}$ is the minimum growth temperature with regards to the growth rate, and b is a scaling parameter. By setting

$$\sqrt{\mu} = a_1 T + a_2, \text{ with } a_1 = \sqrt{b} \text{ and } a_2 = -T_{\min,\mu} \sqrt{b}, \quad (3.12)$$

the growth data as given in 3.3. can be used to obtain values for a_1 and a_2 (and thus for b and $T_{\min,\mu}$) by linear regression, for each separate strain.
For the lag phase there is no generally accepted model available that describes its relation to temperature. However, as suggested by Zwietering et al. (1994) and Wijtzes et al. (1995), based on the fact that Baranyi and Roberts (1994) state that the lag time is inversely related to the μ, we used a reciprocal square root model for the lag phase (see also Ross 1999), p146):

$$\lambda = c/(T-T_{\min,\lambda})^2 \quad (3.13)$$

in which $T_{\min,\lambda}$ is the minimum growth temperature with regards to the lag time, and c is a scaling parameter. By setting

$$1/\sqrt{\lambda} = a_3 T + a_4, \text{ with } a_3 = 1/\sqrt{c} \text{ and } a_4 = -T_{\min,\lambda} /\sqrt{c} \quad (3.14)$$

the growth data as given in 3.3. can be used to obtain values for a_3 and a_4 (and thus for c and $T_{\min,\lambda}$) by simple linear regression, for each separate strain, as for the growth rate.
This procedure renders us two sets of parameter estimates: b, $T_{\min,\mu}$ and c, $T_{\min,\lambda}$. If we have an estimate of N_0 we can predict the concentration for each strain at each time temperature (t,T) combination with the model obtained.

$$\ln(N_t) = \ln(N_o) + b\,(T-T_{\min,\mu})^2\,(t - c/(T-T_{\min,\lambda})^2) \quad (3.15)$$

3.4.2.3.2 Implementation of the data in the models

The fitting procedure outlined above has been applied to the data of the five strains given in Tables 3-8 and 3-9. Fig 3-10 shows the data points and the 'best' fitted curves.
Note that the number of data points per strain is limited. Data points stating 'no growth' cannot be used, as it is unclear whether this is a matter of detection and/or whether μ =0 or λ = infinitely large.
Also note that if the estimates minimum growth temperatures are realistic minimum growth temperatures, $T_{\min,\mu}$ should equal $T_{\min,\lambda}$. This is evaluated in Fig 3-11. It seems that the minimum growth temperatures differ considerably for the psychrotrophic strains z4222 and avtz415, but are more or less equal for the others.

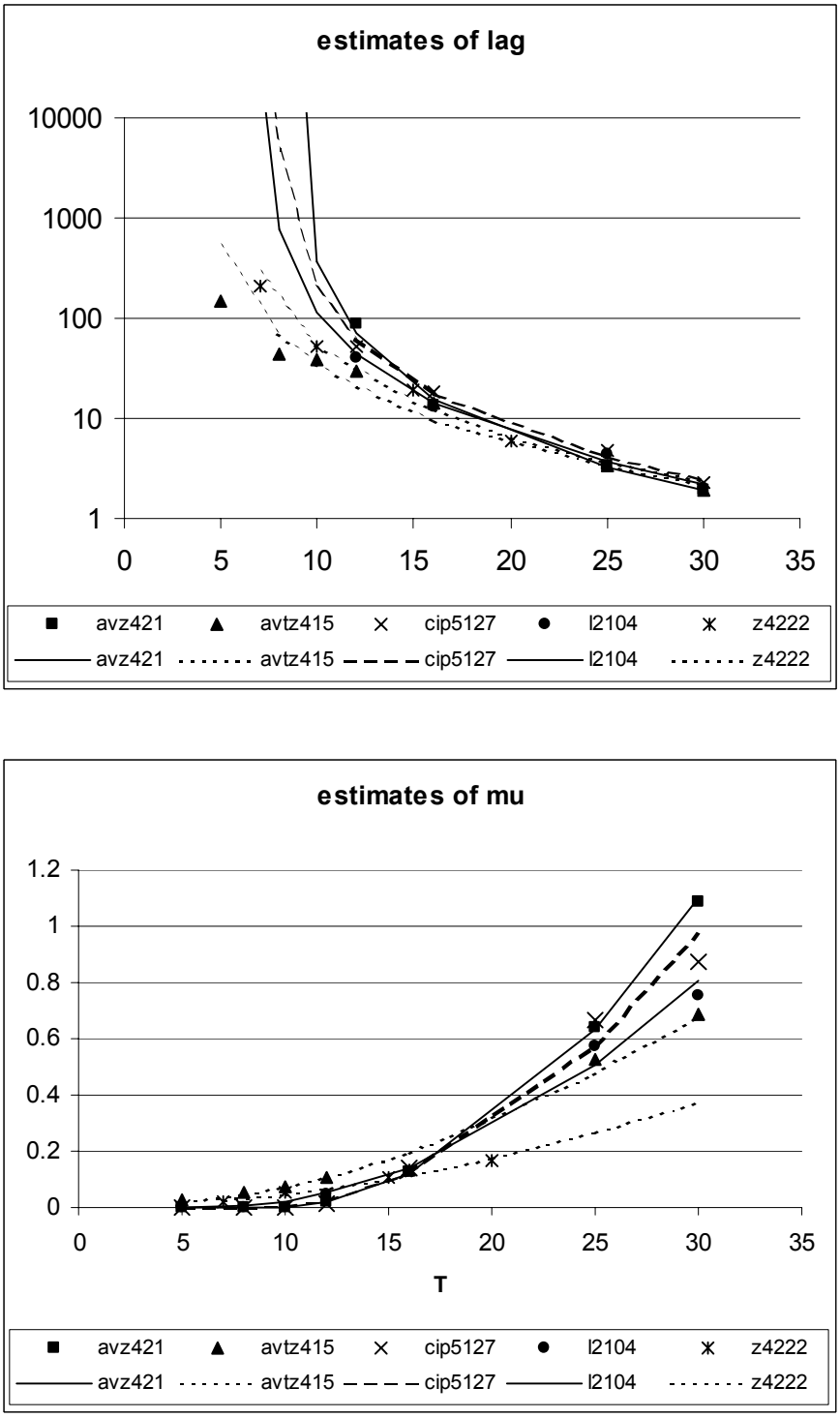

Figure 3-10 Growth data from five different *B. cereus* strains and the fitted curves that results from the fitting procedure outlined in 3.4.2.3.1. Above: lag times plotted versus temperature (°C), Below: maximum specific growth rates versus temperature (°C).

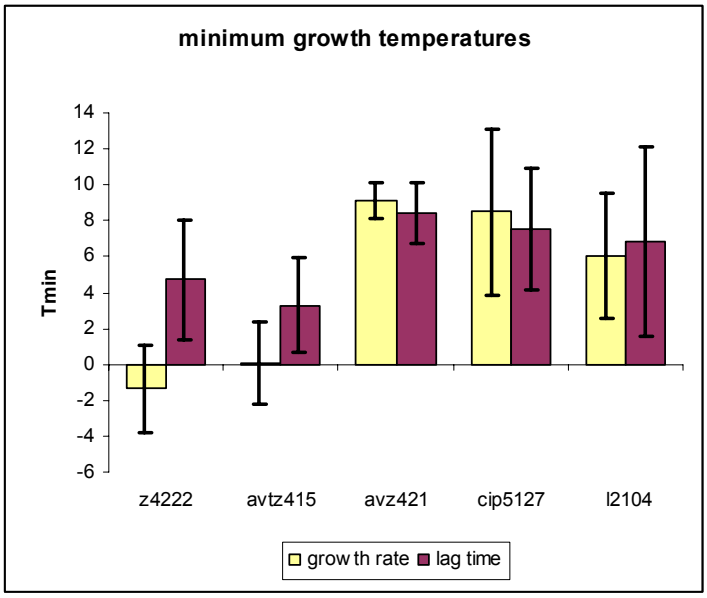

Figure 3-11 Minimum growth temperatures (T_{min}, °C) for μ and λ, as derived from the data. Error bars indicate the 95% confidence intervals.

Next, the uncertainty of the estimate can be implemented by
1) using the mean and standard deviation of a_1 and a_2 (a_3 and a_4) that results from the linear regression of the data fitting procedure in a RiskNormal function, to sample values for a_1 and a_2 (a_3 and a_4) in a Monte Carlo.
2) use these a_1 and a_2 (a_3 and a_4) to calculate estimates of b and $T_{min, \mu}$ and c and $T_{min, \lambda}$ and
3) use these values to estimate the growth curve at temperature T.
In the Monte Carlo simulations this uncertainty has been interpreted as variability between batches.

3.4.2.3.3 The maximum population density

The lag-exponential growth model outlined above (4.2.3.1.) includes a lag phase and an exponential growth phase, but no stationary phase where the maximum population density (MPD) is reached. This is a consequence of the choice for simplicity, and it is not realistic. In part, reaching the stationary phase of the growth curve is not relevant for risk assessment, because this phase is usually associated with spoilage. With spoilage the product is not consumed and therefore it is not hazardous. However, in the current risk assessment the MPD is important for three reasons. First, as stated, if the MPD is reached, this may imply spoilage. If spoilage means that the product is not consumed, it is important to know this, because it reduces the exposure and the risk. Second, if the MPD lies below the spoilage level (say below 10^8 cfu/g), the product may still be spoiled, be it by another microorganism. Third, reaching the MPD may lead to sporulation. If this is so, this may give rise to an increased risk, certainly when the product is not spoiled (see 3.3.5 above).
The level of maximum population density is introduced in the model as a per batch variable with distribution BetaPert(6, 7.5,8.5) log cfu/g based on expert opinion of Frederic Carlin.

When the MPD is reached growth stops and the population density remains constant. With the current values, reaching the MPD does not necessarily imply spoilage by *B. cereus*.

3.4.2.3.4 Dealing with a temperature profile

Like for the inactivation model, the secondary growth model is valid at a constant temperature. In the last part of the food pathway, however, we have to deal with the fact that the temperature changes, while the growth process continues. When the exponential growth phase has been entered, this is easily solved by changing the growth rate to the one that fits to the new temperature. However, when the bacilli are still in the lag phase, the course of events is unclear.

By the lack of a good alternative, we assume here that the lag is summed up with changing temperature: When x% of the lag phase is passed at temperature T_1, the lag phase at temperature T_2 is shortened by x %. This continues until the lag phase has ended and the growth phase starts.

3.4.2.3.5 Non Germination

The 'non germination kinetics' are important to differentiate between spores and vegetative cells at the end of the food pathway. As it is assessed that the germination lag is 0% of the total lag, the onset of growth is sufficient for immediate germination. This 'onset of growth' is assumed to take place when the minimum growth temperature as defined for the lag phase is exceeded.

The probability that a spore does not germinate at growth is estimated to be variable per package, a random sample from a BetaPert(0,0.0001,0.3) distribution (see 3.3.4.).

3.4.2.3.6 Spoilage

Spoilage is important, because usually spoiled products won't be eaten. The incorporation of spoilage in the exposure assessment model is complicated by the absence of a 'general spoilage model'. Clearly, outgrowth of *B. cereus* may occur at conditions that may lead to outgrowth of spoilage organisms too. If we had a general model that predicts (the probability of) spoilage for the product of interest and for a given time temperature profile, this could be compared to growth of *B. cereus*.

However, the data on spoilage show that even the definition of spoilage is obscure: there is no clear spoiled/non spoiled threshold.

As a 'better than nothing' alternative, we use the main characteristic of the square root model for the growth rate to express the relative importance of time and temperature in a 'spoilage model'. Assuming that the minimum growth temperature is 0°C (Zwietering et al. 1996), the square root of the growth rate is linearly related to the temperature. In that case the log of the concentration of a spoilage organism that grows exponentially, will be linearly related to time $\times$ temperature^2, expressed here as tTT. For changing temperature over time tTT stands for the sum of tTT for all time intervals. The data show that spoilage will almost certainly occur after 5 days at 20°C or 20 days at 10°C, that is when tTT = 2000. The situation may be critical

after 10 days at 10°C, that is when tTT = 1000. These two values are therefore used to evaluate the potential occurrence of spoilage of the broccoli puree.

3.4.3 The exposure assessment model

3.4.3.1 The spreadsheet model

The exposure assessment model is set up as a Monte Carlo spreadsheet model in @Risk (Pallisade, Newfield). A single iteration of the model simulates the production, distribution and storage of one batch of broccoli puree and is therefore equivalent to the events along the food pathway of one batch of broccoli puree. Although one batch is used for about 855 packages in reality, in the Monte Carlo simulation only 100 contaminated packages are evaluated in one iteration. With 100 packages we may get an impression of the variability between packages from one batch, whilst keeping the simulation computationally practicable. One run of the model is a sample of 2000 iterations, using Latin Hypercube sampling. This means that one run shows what might happen with a set of 2000 batches.
The model can be run for five different *B. cereus* strains, two psychrotrophic strains, avtz415 and z4222, and three mesophilic strains, avz421, l2104 and cip5127. These strains are considered to be representative for the variability within *B. cereus* (see 3.3.2). The strain specific parameter values, to be used in the inactivation and growth models are given in Table 3-15. When the model is run for a single strain, it is assumed that the prevalence of this strain is one, and other strains are neglected. Doing so, we are able to compare the strains, but we do not regard mixtures of strains.

Table 3-15 Mean values of the growth and inactivation parameters of the five strains evaluated in the model. Numbers in italics are fixed, the others vary per batch. [1] values unknown, adapted from strain avz421.

strain	**growth parameters**				**inactivation**	
	b	$T_{min,\,\mu}$	c	$T_{min,\lambda}$	z	D_{90}
z4222	0.00038	-1.323	1553.248	4.675	*13.3*	*20.4*
avz421	0.00253	9.148	901.915	8.429	8.4	39
avtz415	0.00076	0.036	1571.538	3.275	7.78	2.47
cip5127	0.00208	8.427	1271.258	7.487	8.4[1]	39[1]
l2104	0.00141	6.030	1200.834	6.759	*7.55*	*39.4*

Next, to run the model the consumer fridge temperature profile has to be specified (see 3.4.1.3). The ‘Europe’ profile is the default option, with alternatives ‘South’, ‘North’, ‘Standard’ and ‘Fixed’. In the spreadsheet model all other input values can be changed too, but they are fixed throughout this study.

Based on our findings on the effect of cooking (see 3.5.1. below), it is assumed in the final model that all *B. cereus* spores from broccoli are killed during cooking. So in the 280 kg batch the prevalence P=0. The process then starts with initial contamination of starch and milk protein.
Although in the real process packing (partitioning) takes place before pasteurisation, we model it as if it takes place after pasteurisation. This is done because it is easier to model, while it has no effect on the results, if it is assumed that the effect of pasteurisation is identical for all packages (that is: we are dealing with the same *B. cereus* strain and time and temperature are identical in all packages). In that case distributing cells and killing cells are independent events, and it doesn't matter which happens first.

3.4.3.2 Definition of the model output

In the spreadsheet model the output per iteration is collected on a separate sheet. An example is given in Fig 3-12. The shaded area's are selected as output in @Risk, the values of these are stored, and the relevant statistics are evaluated in another sheet (see Appendix 2).
The important model output per iteration (one batch) is:
a) the mean concentration per package 'before storage', that is before industrial storage, defined as the moment that the growth process starts during cooling.
b) the mean concentration per package 'out fridge' that is at the end of the risk assessment, the moment the consumer takes the product from the fridge.
c) the standard deviation in concentrations 'out fridge' of 100 packages originating from one batch.
d) the percentage of 'risky packages', defined here as the percentage of packages with a concentration between 10^5 cfu/g and MPD, of 100 packages in one iteration.
e) the percentage of packages '>MPD', with a concentration at MPD, of 100 packages in one iteration.
f) the effect of pasteurisation, the log reduction due to pasteurisation and cooling.
g) the percentage of packages with an increase in *B. cereus* cfu, of 100 packages in one iteration.
h) the percentage of 'sterile packages', that the percentage of packages not containing any *B. cereus* as calculated for 855 packages.
i) the percentage of packages containing *B. cereus* spores, of 100 packages in one iteration.
j) The mean concentration of *B. cereus* spores in the packages containing *B. cereus* spores.
k) The maximum T_{min}, that is max[$T_{min,\lambda}$, $T_{min,\mu}$], the temperature needed for growth.

Next, the number of packs (out of 100) that meets one of three criteria is recorded:
1) SBD>0, i.e. the sell by day is passed,
2) tTT>2000, i.e. the sum of time × the square of the temperature is larger than 2000, which indicates "certain" spoilage or
3) tTT>1000, which indicates potential spoilage (see 3.4.2.5).

These data are used to evaluate some conditional probabilities of packages with a critical concentration, e.g. the probability of getting a package with a concentration higher than 10^5 cfu/g, given the MPD is not reached and the SBD is not passed.

log N cfu/g	before storage	-3.64				P(c)	0.09		
log N cfu/g	out fridge	-2.18							
	sd log N out	1.431			P(r)	P(r | c)	0.0000		
	risky packs	0.00%			P(>MPD)	P(c) * P(r)	0.0000		
	>MPD	1%					SBD > 0	tTT > 2000	tTT > 1000
	past. effect	0.41				P(r|crit, c)	0.00	0.00	0.00
	growth	1%			1-P(c)	P(m|crit,c)	0.20	1.00	0.20
mean log(# spores)per pos pack		0.00				P(r|non crit,c)	0.00	0.00	0.00
% pos packs		7%							
		SBD > 0	tTT > 2000	tTT > 1000		P(crit)	5%	1%	5%
# packs	overall	5	1	5	P(crit|c)	P(r|crit)	0.0%	0.0%	0.0%
	risky packs	0	0	0	P(r and crit |c))	P(m|crit)	1.8%	8.8%	1.8%
	>MPD	1	1	1	P(m and crit | c)	P(r|non crit)	0.0%	0.0%	0.0%
	safe	4	0	4	P(s and crit |c)	P(m|non crit)	1.0%	0.9%	1.0%
	risky < 0	0	0	0	P(r and non crit | c)	P(r|nm,crit)	0.0%	0.0%	0.0%
		growth				P(r|nm,non cr)	0.0%	0.0%	0.0%
	Tmin max	5.64							

Figure 3-12 Output sheet of one iteration of the Monte Carlo model, which analyses one batch of broccoli puree, split up in 100 packages. Values in shaded areas are explained in the main text (a – k) and (1-3). For the (conditional) probabilities given at the right, 'c' stands for 'contamination, 'r' stands for 'risky packages', 'm' stands for 'packages where the MPD is reached, 'crate' stands for one of the three criteria, listed (1-3) in the main text.

The mean and standard deviation of the results of 2000 runs are calculated. This gives, among others, the standard deviation of the mean concentrations 'out fridge'. Comparing this standard deviation of the mean (over batches) with the mean of the standard deviations (over packages), gives a clue of the relative importance of 'within' and 'between' batch variability.
The outcomes of the different simulation iterations in a run were compared in correlation plots for four sets of output values:
1) 'before storage' (a) and 'out fridge' (b) to illustrate the relation of the mean concentration after industrial processing to the mean concentration 'out fridge'. The stronger the correlation, the more impact industrial processing has on the mean exposure.
2) 'T,min max' and 'out fridge' to illustrate the relation between the minimum growth temperature and the mean concentration 'out fridge'. The stronger the correlation, the more impact the 'psychotrophic' character of *B. cereus* has on the mean exposure.
3) 'risky packs' and 'before storage' to illustrate the relation between the mean concentration in the product at the end of industrial processing, and the probability of a critical package at the moment the package is taken from the fridge. If the correlation between the two is strong, checking the concentration at the end of processing may be useful.
4) 'risky packs' and 'out fridge' to illustrate the relation between the mean concentration and the moment the product is taken from the fridge, and the probability of a critical package at that moment. If the correlation between the two is weak, variability in storage conditions at retail/and home is the main determinant for the risk.

3.5 Results

3.5.1 Detailed industrial model: The effect of cooking

The cooking process, step 7 in Table 3.1, is analysed separately, to envisage the effect of cooking. With the cooking temperature T = BetaPert (93,98,100), as extracted from the cooking temperature profile (see 3.4.2.2.2), and the inactivation characteristics of different *B. cereus* strains (see 3.3.2) the distribution of the decimal reduction values found in 10000 LHS samples, is given in Fig 3-13.

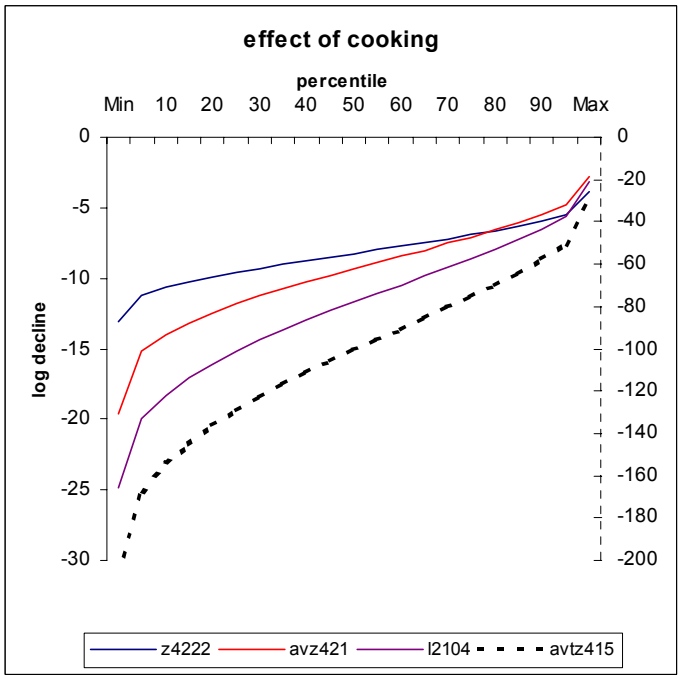

Figure 3-13 The distribution of the effect of cooking for four different *B. cereus* strains. The minimum and maximum values for the log decline and the 5 % percentiles as found in 10.000 LHS model iterations are shown. The three mesophilic strains are represented by straight lines, with the log decline given on the left y-axis. The psychrotrophic strain avtz415 (dashed line) is especially heat sensitive, and is plotted on the right y-axis.

With initial contamination as given (all 400 g broccoli heads contaminated with concentration 10^ BetaPert(-1,0,2) cfu/g) and processes as given, after cooking the main results of 10000 iterations LHS simulation are given in the Table 3-16. It shows that the predicted prevalence (% of contaminated batches) and concentrations are low. Strain avtz415 never survives the heating process. The maximum number of cfu found in 10000 iterations for the 'worst case' strain is about 10^3 cfu per 280 kg batch. This is negligible compared to the contamination by adding the ingredients starch and milk protein, as shown in Fig 3-14. The mean concentration in a batch due to adding ingredients is about 10^6 cfu per batch, compared to less than 5 cfu for the worst case strain after cooking. The minimum number of *B. cereus* cfu in a batch, after

adding ingredients as found in 10000 LHS iterations, is about 2.5×10^3. This is more than twice as large as the maximum after cooking.

Table 3-16 Inactivation of cooking: characteristics of four different *B. cereus* strains as found in 10000 LHS iterations per strain.

strain	mean log reduction	prevalence batch after cooking	max cfu per batch	concentration log cfu/g	mean cfu per batch
z4222	8.3	10.7 %	73	-3.6	0.44
avz421	9.6	15.9%	1032	-2.4	4.63
avtz415	103.7	0%	0		0
L2104	12.1	8.0 %	515	-2.7	1.51

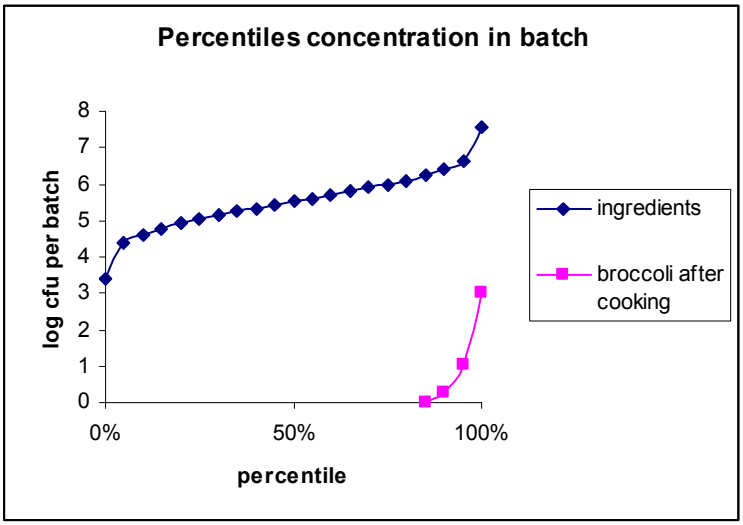

Figure 3-14 Comparison of the contamination on broccoli after cooking, and the initial contamination of ingredients: Even the maximum concentration after cooking is lower than the minimum concentration in the ingredients. As a consequence the contamination on raw broccoli may be neglected; the exposure assessment may start after cooking.

From these finding we conclude that the first part of model can be neglected and that we can use a simplified model focussing on the last part of the food pathway.

3.5.2 Final model

The results of the runs of the spreadsheet model are summarised in Table 3-17 and shown graphically in Figs 3-15 and 3-16. The output sheets are given in Appendix 2.

Clearly, the psychrotrophic, heat sensitive strain avtz415 is the most hazardous strain. If contaminated with this strain, and with the 'South' European time temperature profile, up to 6.4 % of the packages may be contain a level higher than 10^5 cfu per gram. Many of these, however, may be spoiled.
Although its initial concentration in the ingredients is low, and the mean concentration 'out fridge' is relatively low too, the variability between packages from one batch is very large. This results in a wide distribution of concentrations over the packages from one batch. The long 'tail' of this distribution reaches out to the critical area, larger than 10^5. In general, for psychrotrophic strains the mean standard deviation within the batches is much larger than the standard deviation between the means of batches, indicating a large effect of the variability of storage conditions of different packages. In contrast, for the mesophilic strains the standard deviation between batches is larger: variability in storage conditions has little effect because there is less growth.
The percentage of contaminated packages is 100% for the mesophilic strain, but about 65% for the psychrotrophic strain. This is due to the higher initial concentrations of the mesophilic strains.

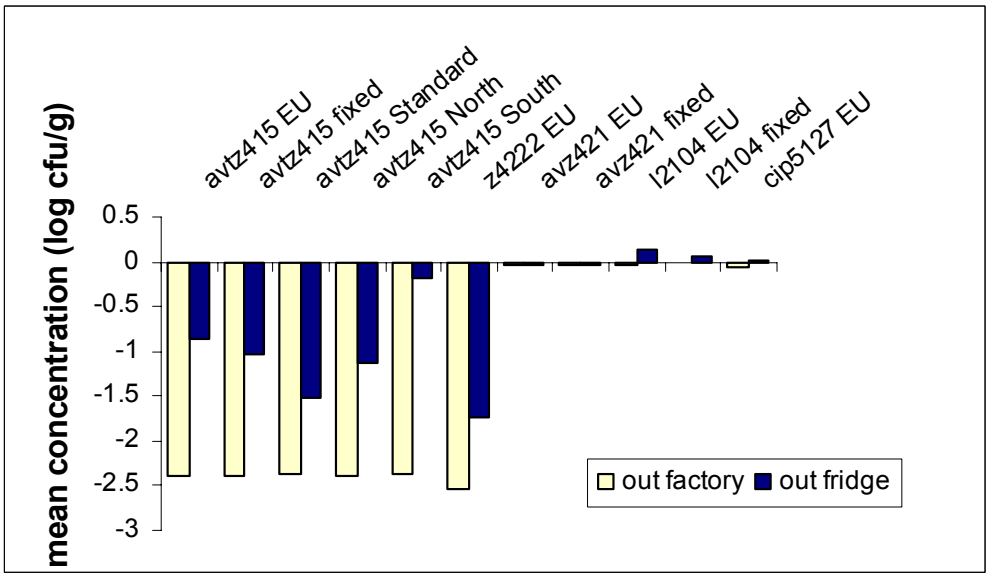

Figure 3-15 Mean concentrations of *B. cereus* in the packages as found in the exposure assessment, for five different strains with different domestic fridge temperature profiles, as given in Table 3-17a. Psychrotrophic strains avtz415 and z4222 show a lower mean concentration, but a larger increase after the product leaves the factory. The fact that for psychrotrophes a larger percentage of packages contain high concentrations (see fig 3-16) is due to a much larger variability between packages.

Table 3-17a Results of the exposure assessment model. For five different strains and for different domestic fridge temperature profiles, several results are listed: mean log concentration before storage, after step 12; the mean log concentration 'out fridge', after step 16; the mean standard deviation of log concentrations of 100 packages per batch; the standard deviation of the mean log concentration per batch; the mean percentage of contaminated packages; and the mean percentage of packages containing *B. cereus* spores.

strain + fridge temp. profile	before storage (log cfu/g)	out fridge (log cfu/g)	sd out within batch	sd out between batches	contami-nated packages %	packs with spores %
avtz415 EU	-2.39	-0.86	2.32	0.96	68	16
avtz415 fixed	-2.39	-1.03	1.73	1.02	68	16
avtz415 Standard	-2.38	-1.51	1.29	0.79	69	17
avtz415 North	-2.39	-1.14	1.83	0.91	69	16
avtz415 South	-2.37	-0.18	3.21	1.09	69	17
z4222 EU	-2.53	-1.74	1.23	0.59	62	15
avz421 EU	-0.04	-0.03	0.16	0.68	100	92
avz421 fixed	-0.03	-0.03	0.04	0.68	100	90
l2104 EU	-0.03	0.13	0.54	0.76	100	91
l2104 fixed	-0.02	0.06	0.19	0.72	100	90
cip5127 EU	-0.05	0.02	0.36	0.70	100	91

Table 3-17b Results of the exposure assessment model –continued-. For five different strains and for different domestic fridge temperature profiles, several results are listed: [1]the percentage of packages in which *B. cereus* has grown; [2]the percentage of packages with a concentration higher than the critical level (10^5); [3]the percentage of packages with a concentration between 10^5 and MPD, of those packages in which the MPD is not reached; [4]the percentage of packages with a concentration between 10^5 and MPD, of those packages in which the MPD is not reached and the SBD is not passed; [5]the percentage of with a concentration between 10^5 and MPD, of those packages in which the MPD is not reached and tTT < 1000 (potential spoilage threshold); [6]and the percentage of with a concentration between 10^5 and MPD, of those packages in which the MPD is not reached and tTT < 2000 (certain spoilage threshold), all at the end of the food pathway.

strain + fridge temp. profile	growth[1] %	P(conc>10^5)[2] %	P (X)[3] %	P (X | < SBD) [4]%	P (X | tTT <1000)[5]%	P (X | tTT <2000)[6]%
avtz415 EU	30	3.5	1.6	1.2	0.6	1.5
avtz415 fixed	30	2.2	1.2	0.7	0.6	1.1
avtz415 Standard	20	1.0	0.6	0.5	0.3	0.5
avtz415 North	27	2.2	1.1	0.8	0.5	1.0
avtz415 South	38	6.4	2.5	2.0	0.8	2.3
z4222 EU	15	0.7	0.3	0.2	0.0	0.2
avz421 EU	4	0.1	0.0	0.0	0.0	0.0
avz421 fixed	2	0.0	0.0	0.0	0.0	0.0
l2104 EU	17	0.7	0.3	0.3	0.1	0.3
l2104 fixed	16	0.2	0.1	0.1	0.1	0.1
cip5127 EU	8	0.3	0.2	0.1	0.0	0.1

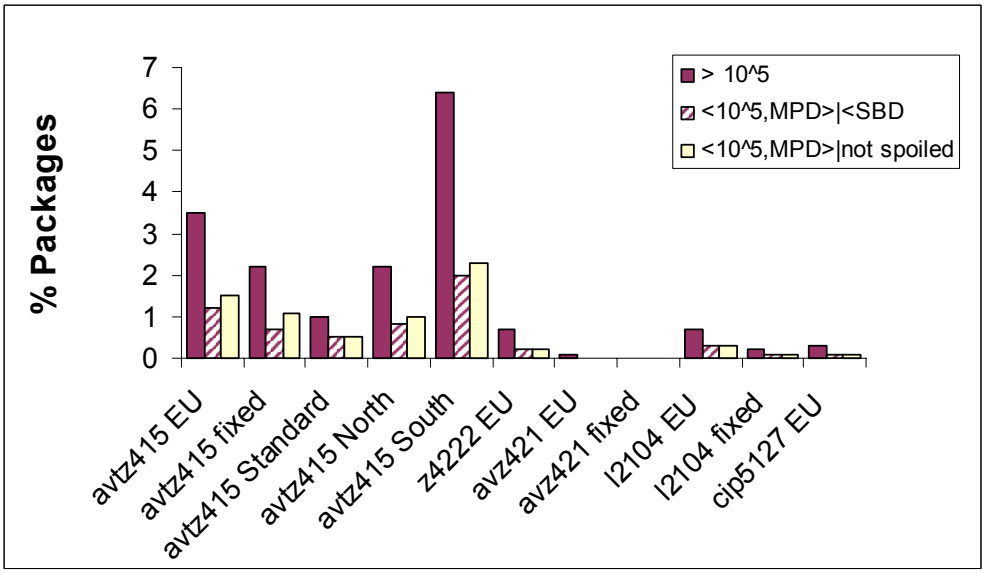

Figure 3-16 The percentages of packages containing critical levels of *B. cereus*, as given in Table 3-17b in columns [2],[4] and [6]. Percentages of packages with more than the critical level of 10^5 cfu/g are adapted for reaching MPD, passing SBD and spoilage. Apparently the psychrotrophic strain avtz415 with the 'South' domestic fridge temperature profile holds the largest risk.

As expected, the 'South' temperature distribution of home fridges yields a higher risk than the mean 'Europe' distribution. Note however that even the 'Standard' distribution, that is similar to the international standard, holds some risk, even if the SBD is respected. This 'Standard' is safer than a temperature 'Fixed' at 7°C, which apparently may be to high for psychrotrophic strains.

As mentioned in 3.4.3.2. some correlation plots were constructed to compare the results of different model outputs of the iterations in a model run. Illustrated in Figs 3-17 and 3-18 are the correlation plots as found for a psychrotrophic and a mesophilic strain with the EU home fridge temperature profile. The psychrotrophic strain shows intermediate correlations in each graph, and the mean concentration before storage apparently has little predictive value for the situation at the moment the consumer takes the product from the fridge. The mesophilic strain show a strong correlation between the mean concentrations 'before storage' and 'out fridge'. There is, however, no correlation with risk.

The percentage of packages containing spores is much higher is mesophilic strains. Thus, if not the 'cfu' but the spores are the actual hazard, it may be that mesophilic strains are the hazardous and not psychrotrophic strains.

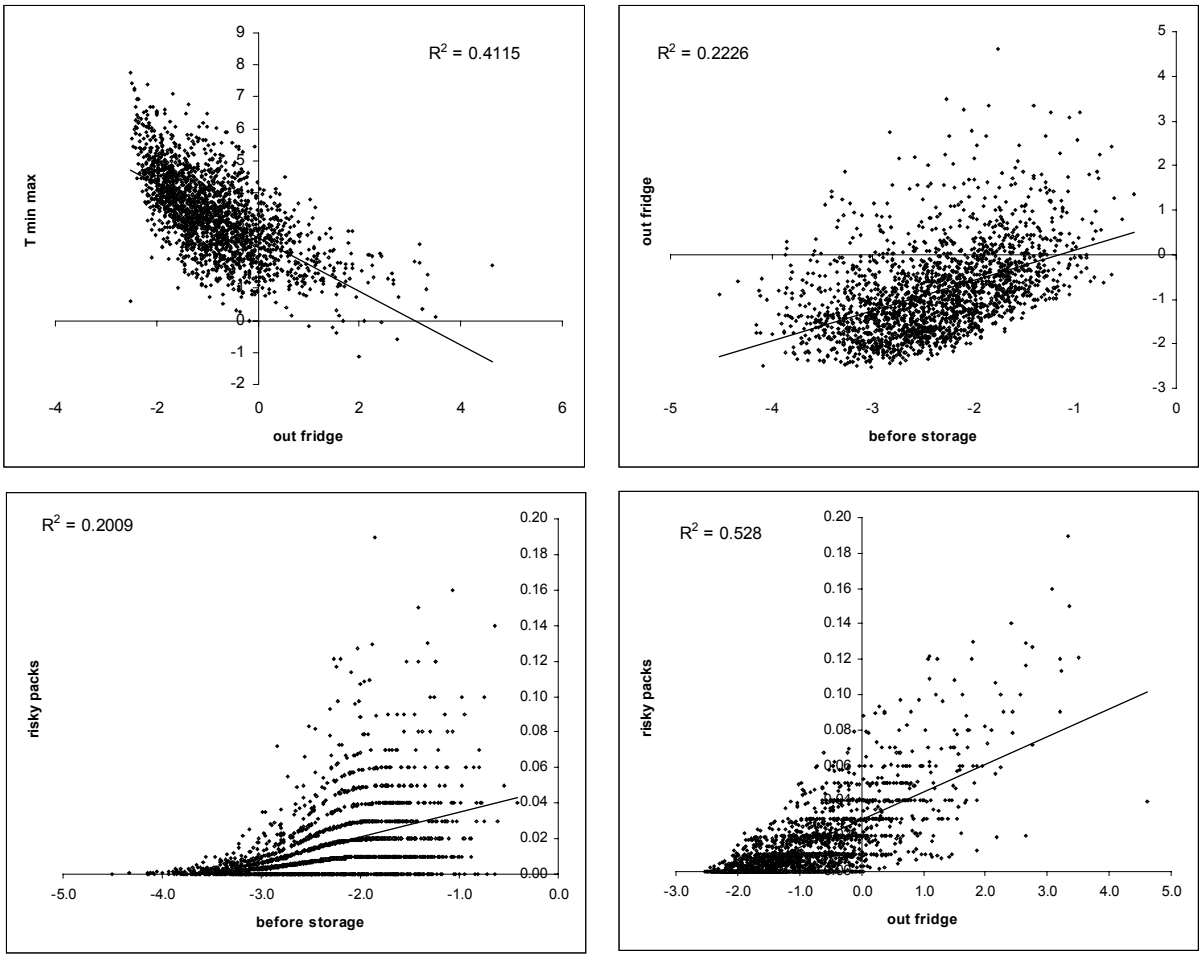

Figure 3-17 Correlation plots of the results of the exposure assessment for the psychrotrophic strain avtz415 with the 'Europe' domestic fridge temperature profile. The output of two different parameters is plotted for 2000 model iterations. Above left: The mean 'out fridge' concentration (log cfu/g) and the maximum T_{min} (°C). Above right: The mean 'before storage' concentration and the mean 'out fridge' concentration. Below left: The mean 'before storage' concentration and the percentage of 'risky packs'. Below right: The mean 'out fridge' concentration and the percentage of 'risky packs'. The discrete fractions of risky packs are a consequence of sampling only 100 contaminated packages per batch in the simulation. The value of the correlation coefficient R^2 is added as an indication of the degree of correlation between the plotted variables

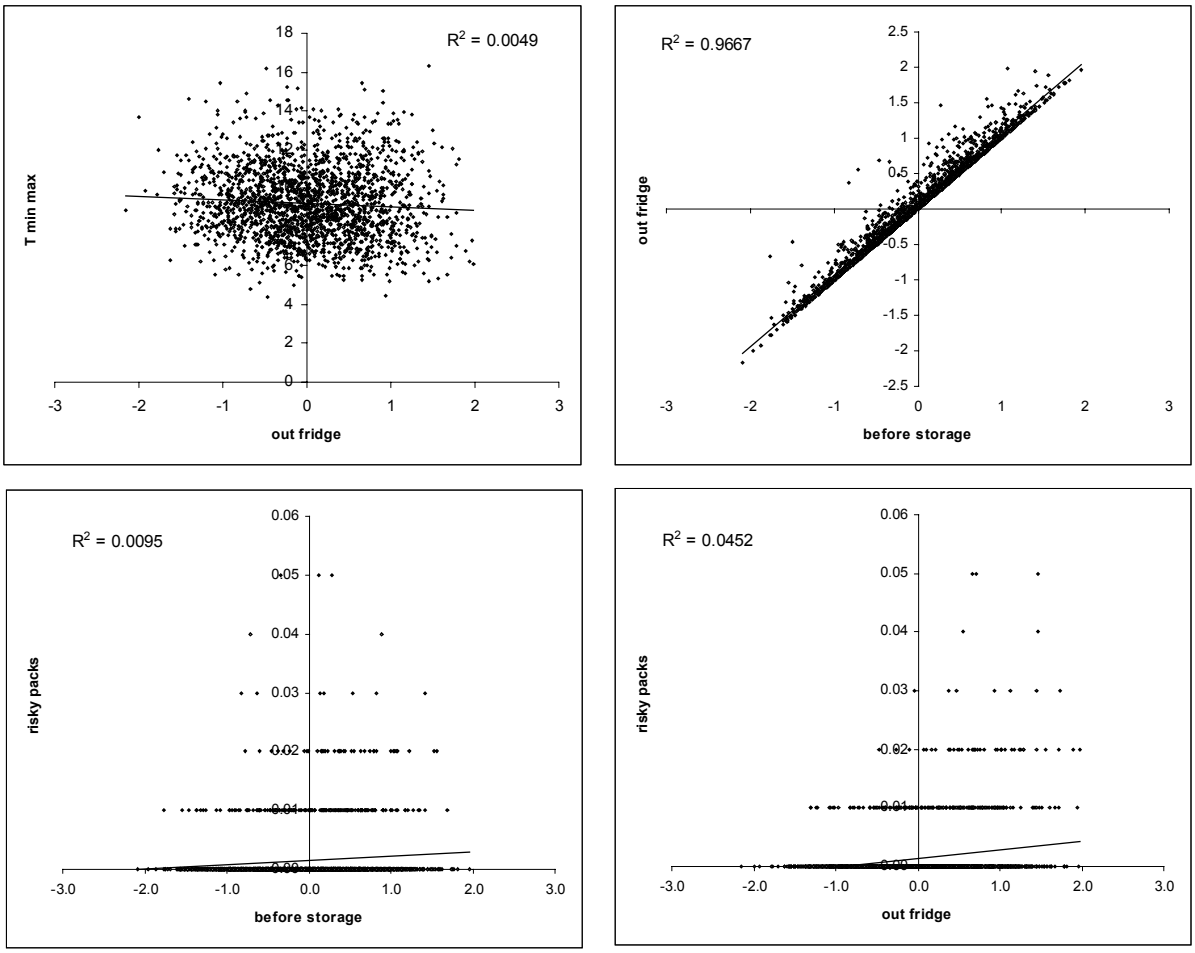

Figure 3-18 As 13-17, correlation plots of the results of the exposure assessment for the mesophilic strain cip5127 with the 'Europe' domestic fridge temperature profile.

3.6 Discussion

3.6.1 Results of the *B cereus* in broccoli puree exposure assessment

3.6.1.1 The exposure to B. cereus in broccoli puree

The results of the Monte Carlo simulation with the QMRA model indicate the following:
- The *B. cereus* contamination of packages broccoli puree originates from the ingredients, not from the broccoli.
- If the initial contamination is with mesophilic *B. cereus*, all packages are contaminated, if contamination is with psychrotrophic strains, about 65% is contaminated.
- Although the initial level of contamination with psychrotrophic *B. cereus* strains is assumed to be two log units lower than that of mesophilic strains, psychrotrophes give rise to a higher percentage of packages above the critical level at the endpoint.
- For psychrotrophic strains the variability between packages that originate from one factory batch is larger than the variability between the means of batches. For mesophilic strains this

is the other way around. This difference is caused by a higher frequency of growth of psychrotrophic strains. As a result mean *B. cereus* concentration in packages at the factory is a bad predictor for a critical concentration at the endpoint.
- The percentage of packages with a critical concentration larger then 10^5 cfu/g at the moment the consumer takes the product from the home fridge may range from 0 to 6.4%. This includes spoiled packages. This percentage is largest for the psychrotrophic, heat sensitive strain avtz415, with a 'south European' home fridge temperature profile.
- By the lack of an appropriate 'spoilage model', it was difficult to assess the percentage of critical, non spoiled packages of broccoli puree. The percentage of packages with a critical concentration that does not lie at the maximum population density ranges from 0 to 2.5%. Keeping to the SBD is only a little helpful, as the range is than between 0 to 2.1%. The percentage of packages with a critical concentration that does not lie at the maximum population density with a temperature profile tTT<2000 ranges from 0 to 2.4%, with a temperature profile tTT<1000 it ranges from 0 to 0.7%.

3.6.1.2 The impact of the exposure assessment model

The risk model results indicate that there is a probability of ending up with a critical concentration in the broccoli puree packages. The discussion about the impact of this result may centre around two points:

3.6.1.2.1 The reliability of the model estimates

The reliability of the model output depends on both the reliability of the input values, and the reliability of the models used.
In the risk model, all probability distributions are interpreted as variability, either between packages or between batches. Uncertainty is not modelled. As a consequence, the model output does not give any uncertainty intervals. The results, presented as 'probability of critical concentrations', are point estimates. We have no quantitative expression of their reliability.
As the differences between strains can be interpreted as 'uncertainty about the initial *B. cereus* strain', some uncertainty is included in the model. This is, however, only one aspect of the total uncertainty. Uncertainties not incorporated include uncertainty about the range of initial contamination, uncertainty about the actual time temperature profile during retail and for consumer behaviour, and, probably the most important, model uncertainty. The inactivation model is somewhat uncertain as its predictions are not checked in the product itself. The growth model predictions are very uncertain, because the model holds many assumptions and is not validated in the product. Critical assumptions in the growth model are the 'inverse square root' model for the relation between lag and temperature and the summation of lags as applied with changing temperature. The latter assumptions are so hypothetical, that we are not able to assess their attending uncertainty. Although quantification of minor sources of uncertainty in some parts of the food pathway may be

possible, the expectation that it will be overwhelmed by the uncertain uncertainty during growth, led to the decision not to incorporate uncertainty at all.
The results of the risk model should be regarded with the uncertainty in mind. The model estimates are the estimates that are the result of expert opinion where data are lacking and of the best models that we could find. All assumptions, data and models are thoroughly discussed during several meetings of the RASP project. Therefore the results are to be regarded as the logical result of the knowledge and 'best guesses' of a group of European experts. They reflect the state of the art of food microbiology in the context of microbial risk assessment of sporeforming pathogens.

3.6.1.2.2 The relation between critical concentrations and health risks.

The endpoint of the risk assessment model is the moment the consumer takes the product from the refrigerator. At this moment, a concentration of 10^5 cfu/g is taken as the critical level (Notermans et al. 1997).
This endpoint is not the moment of consumption. The effects of additional heating of the product by the consumer is not included in the model, because we did not have sufficient information on that. Other things that the consumer can do with the product (like storage of left overs, reheating etc.) are omitted too. Strictly speaking, the exposure assessment therefore does not predict exposure defined as "the probability distribution of ingested organisms".
The concentration evaluated is the concentration of 'colony forming units'. These cfu's, however, are probably not the hazard of interest. The real hazard of *B. cereus* is not the organism itself, but (one of) its toxin(s). Not all strains are toxigenic, and the dynamics of toxin formation are largely unknown. Also, there may be a large difference between spores and vegetative cells, which are both 'cfu's'. Spores may be the major hazard, because the survival of spores and cells in the stomach will be very different. A low dose of spores (below the critical level) may pose a higher risk than a high dose of vegetative cells when vegetative cells are inactivated in the stomach and spores are not.
Our model results indicate that for the psychrotrophic strains about 16% of the packages contains spores, and for the mesophilic strains about 90%. The interpretation of these results is complex. Many of these spore containing packages contain spores that are not germinated during storage or transport, as a direct consequence of information provided (see 3.3.4.). It is unknown whether such spores will germinate in the intestinal tract after ingestion. If not, they may be considered 'dead', and do not pose a risk. In contrast, however, potential sporulation after reaching the MPD is neglected. The model results show that this may occur in up to 4% of the packages. This may be interpreted as a major source of risk for the consumer, because many spores may be formed, which need not be inactivated by potential consumer heating. However, this is dubious on its turn because reaching the MPD will often be associated with spoilage, and spoiled products are (probably) not consumed. It is, in short, very difficult to interpret our numerical results as quantitative indicators of health risks.
For all these reasons the quantitative outcome of the risk assessment model should not be read as 'percentages of broccoli puree packages that are a threat to public health'. Not only is

the estimate uncertain, its relation to public health is doubtful too. However, the relative outcome of the estimates for the different strain types and the home fridge time temperature profiles is of interest, because it gives insight in the importance of these aspects for the evaluation of the safety of a minimally processed vegetable product. Although we are not able to quantify the risks, we can make proposals for effective risk mitigation strategies.

3.6.1.3 Proposals for risk mitigation

An important objective of risk assessment is the evaluation of risk mitigation strategies, by estimating the effects of 'alternative scenario's'. In the current risk assessment model, we focussed on the impact of keeping to the sell by date on the packages, and the international 'standard' temperature profile. It shows that keeping to the SBD is not sufficient: this has a minor impact on the percentage of packs with a critical level of *B. cereus*, because the effect of temperature abuse may be larger than the effect of 'time abuse', which on its turn is the consequence of the square root relationship between temperature and growth rate. With 'ideal' home refrigerators the number of packages with a critical level will lower. A better control of home refrigerator might therefore be a possible risk mitigation strategy, although it may be difficult to realise this. Note in this context that the data on domestic refrigerator temperatures show that assuming that these refrigerators keep to the international standard, or assuming temperatures below 7°C is not realistic. Even the 'North' European temperature profile, the 'best one' available from the data, does not meet the results international 'Standard'. Extended research and improved public information may be necessary here.
Although not investigated in detail, a clear alternative risk mitigation strategy at the factory will be to lower the initial concentration in the ingredients, that are the source of the *B. cereus* in the end product. How to realise this lower concentration is a practical issue that falls beyond the scope of this study. Clearly, the contamination of the broccoli is not relevant when (almost) all spores are killed during the cooking process.
Sampling at the endpoint of the food production process to check for a critical level of *B. cereus*, appears to be a rather useless control measure. This is illustrated in Fig 3-16. The fact that the variability between packages is large, results in a small correlation between the mean concentration at the end of processing, and the probability of a concentration above the critical level at the moment the consumer takes the product from the refrigerator. If a package has a high concentration of *B. cereus*, this is little informative about the concentration in other packages that originate from the same batch. High concentrations are mainly the consequence of time temperature abuse, not so much of a high initial concentration.

3.6.2 Microbiology and risk assessment

3.6.2.1 General considerations

In QMRA, microbiological expertise and microbiological data have to be integrated with a risk assessment model. As outlined above, for the construction of a risk assessment model, the food pathway has to be described in terms of unit sizes, time and temperature. By applying the MPRM framework by addressing basic processes to separate processing steps, a line of models can be set up, to quantitatively describe the transmission of the hazard along the food pathway. Next, microbiological expertise and microbiological data have to be integrated with a risk assessment model. This is not as easy as it may seem, for several reasons.
Traditionally, microbiology is a discipline with limited attention for quantitative aspects. The results of microbiological experiments are formulated in terms that differ significantly from what is needed as input for risk assessment models. Similarly, risk assessment may have severe difficulty with dealing with microbiological processes and variability. As risk assessment is a new discipline within microbiology (and microbiology is a new discipline in risk assessment), it now appears that there are large gaps in microbiological knowledge, which are crucial for risk assessment. Examples that we ran into during this study are quantitative knowledge of spore germination and sporulation, the effects of non- isothermal processes on growth and inactivation, predictions of variability and quantification of uncertainty in growth and inactivation models.
In our view, the bridging of the gap between microbiology and risk assessment will be one of the major challenges in microbial risk assessment in the near future.

3.6.2.2 Inactivation modelling

Along the food pathway, inactivation is important during the industrial processing steps ‘cooking’; and ‘pasteurisation’. Inactivation is modelled using the well known loglinear Bigelow model. The number of inactivation models to choose from is large(Van Gerwen and Zwietering 1998, Cerf et al. 1996 , Xiong et al. 1999). We selected the simplest model that incorporates the effects of time and temperature, for which parameter estimates were available from within the RASP project. Note that the estimates for D and z are not derived from experiments with broccoli puree, and may therefore not be a precise predictor of inactivation in this food product. We had, however, no alternative, and no insight in how to adapt the parameter estimates for our purpose.
An important aspect of the Bigelow model is that it assumes isothermal inactivation. In contrast, both the cooking process and the pasteurisation process are non-isothermal. For the pasteurisation process, equivalent terms for inactivation at 70°C were supplied by the food manufacturer. For the cooking process, we had to create our own model, as presented in 3.4.2.2.2. This model is currently being tested by A. Fernandez (IATA, Spain). She exposed

spores of the strain avtz415 to the temperature profile and found no surviving spores, thus confirming our prediction (but not necessarily validating our model). Other experiments are in progress (*unpublished results*).

3.6.2.3 Growth modelling

For the risk assessment we have to predict growth at a (set of) temperature(s) for a (set of) storage time(s) for all *B. cereus* strains that might be present on broccoli. Models that are publicly available (Zwietering et al. 1996, USDA 1998) are difficult to implement in a @Risk spreadsheet model and, more importantly, do not include the variability between different types of *B. cereus*. A 'worst case' model is insufficient for QMRA, because it may wrongly suggest to be 'fail safe', and does not give a clue on the probability of ending up with an undesired situation.

To circumvent the problem of finding an appropriate model from the literature, we used growth data acquired within the RASP project to model growth. This has the advantage that we can model an array of strains, from which we have both growth and inactivation data available. These strains are considered representative for the range of *B. cereus* strains found in vegetables. However,, we have to realise that:

1) the data are not about growth on broccoli or in broccoli puree
2) the data are collected in an experimental set-up, once or twice per strain. Presumably a lot of variability is not visible in the data.
3) the data only reflect a few temperatures, we have to intra- and extrapolate
4) the data are collected in isothermal conditions and applied to a non-isothermal process.

The available data were fitted to a simple growth model, to predict the increase with time and temperature. This model has not been validated. The fact that the model holds two values for minimum growth rate ($T_{\min,\lambda}$ and $T_{\min,\mu}$) is difficult to interpret in reality. The relation between these model parameters and the actual minimum growth temperature is obscure. However, if these two 'minimum growth temperature' parameters are set equal, the resulting model could not be fitted to the available data at an acceptable level. Next, the applied procedure of fitting the data to the model was somewhat 'fuzzy' because the original data were not available. The estimates μ and λ from the data in Tables 3-8 and 3-9 should in fact have been obtained with the with lag-exponential model and not with the Baranyi model. Now we don't fit the proper estimates in the model. Ideally, the raw data should be fitted with the model

$$\ln(N_t) = \ln(N_o) + b\,((T-T_{\min})^2\,t - c) \qquad (3.19)$$

so we can directly estimate three parameters, among which only one $T_{\min}$.

The 'inverse square root model' that we used for the lag time is hypothetical and was chosen by the lack of an alternative. This model was tested for *Lactobacillus curvatus* by Wijtzes et al. (1995), who concluded that the model describes the trend well. Ross (1999) mentions the model, but does not dwell on it. He uses the lag generation time ratio (LGR) to derive distributions of the lag time. This LGR typically has a most likely value between 3-6, and has

an upper 95% percentile of about 10-15 generation times. In the terminology used here the LGR = $\mu.\lambda/\ln(2)$. For the data used here this LGR has a mean 3.2, and a range 0.5-6.6. This indicates that the values found are within the range to be expected, but it also indicates that the assumption that $\mu\lambda$ is constant is not met. This implies that the predictive value of the model can be doubted.
Another invalidated assumption is that lag in non-isothermal conditions can be summed up. This, again, is probably not correct, especially with large temperature changes. The idea behind the lag phase is 'a period of adaptation', and with changing conditions the cells will have to adapt continuously. By the lack of an alternative, the 'sum up' model is chosen for its simplicity, in consultation with the experts in the RASP project.
The risk assessment requires a model that predicts growth of *"B. cereus"* for any time temperature profile. As "*B. cereus*" is a collection of strain types with very diverse characteristics, variability in growth characteristics between strains, as found in different experiments, has to be incorporated in the model.. For that purpose, we choose a selection of representative strains. Given the lack of alternatives, we consider the growth models used in this study as the best choice so far.

3.6.2.4 Sporulation and Germination

The different states of *B. cereus*, as spores or vegetative cells, are a complicating factor. Whereas inactivation models of *B. cereus* predict the fate of *spores*, growth models predict the fate of *colony forming units*, that is *spores and vegetative cells*. Usually growth experiments with *B. cereus* (as used for developing growth models) start with spores and measure concentrations of vegetative cells and spores as a function of time. However, strictly speaking this is a combination of germination (from spore to vegetative cell) and growth (from vegetative cell to vegetative cells).
The difference between the cell states modelled by growth and inactivation models implies that if growth is followed by inactivation, and we want to assemble these two in one model, we need a model to quantify the number of spores among the colony forming units. Without such a model, we calculate the survival of vegetative cells as if they were spores. For sporeforming pathogens we therefore not only need growth and inactivation models, but also sporulation and germination models. This is illustrated in Fig 3-19. If a growth process is followed by inactivation, we need to predict the number of spores that we end up with. These spores can be the product of both 'non-germination' and sporulation. Quantitative models to predict this are not available to our knowledge, nor could we find any quantitative data on this. The lack of sporulation models is one of the 'gaps' between microbiology and risk assessment identified in this study.
Fortunately, in the food pathway studied here, growth could be neglected before the inactivation steps, so that we only had to deal with growth after inactivation: We started with modelling spores and ended up with colony forming units. We could model the food pathway without a precise 'germination' model, because the applied growth model implicitly includes germination. To differentiate between spores and vegetative cells in the cfu that we end up

with, we used a very simple ‘non-germination’ model, which states that a variable number of spores will not germinate during growth, and that the ‘germination lag’ is extremely short. This model is chosen for its simplicity and on the basis of expert knowledge. Of course it should be validated if it has to applied again in the future.

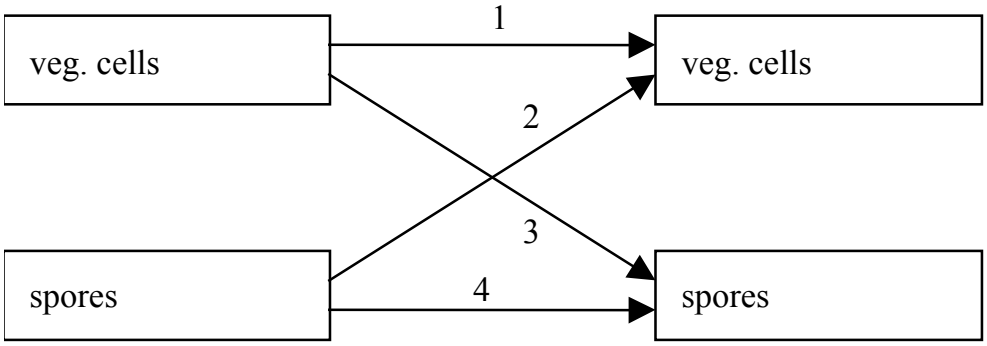

Figure 3-19 The possible transitions of spores and vegetative cells: 1 = growth (or inactivation), 2= germination, 3= sporulation, 4 = inactivation. Available models describe transition 1 (inactivation) or a combination of 1 and 2 (“growth”). For process risk modelling, where different models are linked, models are needed for all four processes separately.

3.6.2.5 Spoilage

Spoilage is a complicating factor in the risk assessment of foods for three reasons. At first, people generally don’t eat spoiled products. Second, spoilage and risk will be correlated because foods with critical doses of a pathogen have often been exposed to conditions that may lead to spoilage probably with an other microorganism). If foods with a critical level of pathogen are not eaten, they pose no risk to public health. Third, spoilage may lead to a change of growth conditions, thus influencing the growth dynamics of the pathogen.
For a proper risk assessment, we need a spoilage model that predicts the probability of spoilage of a given product, given its time temperature history, and initial contamination by ‘spoilage organisms‘. To our knowledge such a model is not available. Modelling spoilage is complicated by the large number of possible spoilage organisms, which may have very different growth characteristics, and contaminate the initial product with variable concentrations. A general spoilage model for a specific product might be obtained by collecting spoilage data at a set of conditions that are relevant for risk assessment. A precise definition of spoilage is needed, which is ideally expressed in terms of probability of consumer acceptance as a function of time and temperature: The percentage of products that are eaten although ‘smelling badly’ will be larger than the percentage eaten when ‘visibly spoiled’. This probability of consumption as a function of the time temperature history of the product, is the output of interest for risk assessment.
In the current model we evaluate spoilage by regarding the MPD of *B. cereus* and the ‘tTT’profile. When the MPD is reached, the concentration of *B. cereus* is high, between 6 and 8.5 cfu/g (see 3.4.2.3.3). This may imply spoilage by *B. cereus*, but it need not. As the conditions responsible for reaching the MPD will be conditions that favour spoilage, the

probability of spoilage given that the MPD is reached, may be large. As an alternative, we look at the 'tTT' profile as an indicator of spoilage. As explained in 3.4.2.5., the threshold tTT = 2000 is regarded as a 'high probability of spoilage' value, and tTT = 1000 is regarded as a 'low probability of spoilage' value. This very simple model is based on some simple arguments, and is of little value without improvement and validation. This model shows how a spoilage model can be used in a risk assessment, and gives a clue about the potential impact of spoilage, given some basic assumptions, based on scarce data. As an example, the results of avtz415 'South' show that large probability of packages with concentrations of *B. cereus* above the critical level (6.4%), is associated with a high probability of spoilage (10.7% tTT>1000), and a relatively high 'relative risk', expressed as the probability of a concentration > 10^5, given the MPD is not reached and tTT<1000, of 0.7%.

4. Evaluation of the MPRM

This report combines the description of a QMRA framework, the modular process risk model (MPRM, chapter 2), with a specific exposure assessment of *B. cereus* in broccoli puree (chapter 3) that was part of the RASP project. The main objective of this exposure assessment was to illustrate the methodology proposed and to examine its utility.
The seven consecutive steps of the MPRM are outlined in paragraph 2.2, and are followed in the exposure assessment in chapter 3.
1) The statement of purpose is given in 3.1. In the progress of the RASP project it appeared of major importance that the statement of purpose is defined precisely. Originally, this statement was much broader than defined here. It appeared however impossible to perform a QMRA on 'sporeforming pathogens in REPFEDs' in general. When the purpose of a risk assessment is not restricted to a well defined hazard, product, food pathway combination, as depicted in Fig 2-1, it is not possible to construct a MPRM. For general questions on the risk of a collection of hazards or a collection of products and food pathways processes, one either has to make generalising assumptions and supply generalised answers, or has to construct a MPRM that is far more complicated than the one outlined here.
If the risk assessment is part of a complete risk analysis, as defined in the CODEX guidelines (1998), the interaction with risk management is crucial. An independent risk manager should supply the statement of purpose for the risk assessment. Communication about this statement of purpose is important, because this statement should supply a question that is both relevant for the risk management and answerable by the risk assessment. If the risk assessor expects a very uncertain answer, this should be made explicit beforehand.
2) The food pathway is described in 3.2.1 and 3.2.2. It is crucial to have a description of the production process that is representative for the product of concern. If QMRA is compared with a Christmas tree, the food pathway is the tree. With the description of the food pathway, one has to keep the purpose of modelling of the transmission of the hazard in mind. 'Secrets' in the food production process that are not relevant for transmission modelling (like for example the names and quantities of sterile spices) may be left out, if this is preferred by the food manufacturer. The description of the food pathway at the retail/consumer phase is important when the exposure his to be evaluated at the level of the consumer. As data are scarce here, simplifying assumptions had to be made in our case study. In general, data on consumer behaviour are a major gap in knowledge. For a proper QMRA, especially for public health purposes, both processing information and consumption data are however indispensable.
3) The MPRM model structure is built in 3.2.3. It appears we only used three basic processes in the final exposure assessment model : growth, inactivation and partitioning. Unfortunately, mixing, removal, cross contamination and ‘black box’ models are not applied. This was not foreseen at the start of the research project. In the initial food pathway, the industrial processing includes removal (of ‘dirty’ broccoli trunks at the start of the process step 3 of Table 3-1) and mixing (of broccoli trunks in a batch, step 5 in Table 3-1). As given in

paragraph 2.3.2., models for these processes were developed, but after it appeared that broccoli contamination was not important for the exposure assessment (paragraph 3.4.1.), they are left out of the Monte Carlo model. This reduced the complexity of this model but had very little effect on the outcome (*data not shown*).

4) An overview of the data used in the exposure assessment is presented in 3.3. This particularly concerns the microbiological data on initial contamination, inactivation, growth, sporulation and germination, and spoilage. Note that there is a discrepancy in the availability of data. Inactivation and growth are well studied. Although implementation of the data concerning these aspects is not straightforward, the amount of data on these topics is very large, compared to the (quantitative) data on initial contamination, sporulation, germination and spoilage. In the case study it appeared that the data on initial contamination of both the broccoli trunk and the ingredients was scarce, and had to be based on expert opinion. Still, this first input in the risk assessment is very important. In the present study most variability in the model output is explained by the broad range of 'variability' in the initial contamination of the ingredients, which is highly uncertain. It should be possible to gather more and better information on initial contamination. Doing so, one should try to quantify both the uncertainty and the variability in the estimates, collect information on the prevalence and concentration of different strains (e.g. are there mixtures of strains? Of psychrotrophes and mesophilics?).

5) The selected models used for the different basic processes are presented in 3.4.1. and 3.4.2. In general, we use simple models. An important reason for that is that we are not so much interested in a precise point estimate, but merely in a prediction that includes the variability and uncertainty of growth and inactivation. This is complicating enough as it is.

6) The microbiological data are combined with the model in 3.4.2. It is evident here that there is a gap between risk assessment input and microbiology output. Risk assessment needs distributions, not means and standard deviations. Microbiology deals with big sources of variability and uncertainty, and risk assessors have to incorporate that in their models.

7) The exposure assessment is performed by running the constructed model in a spreadsheet, as explained in 3.4.3.

So far, The MPRM structure is not yet fully developed. Ideally, we should have a plain overview of the basic processes and their possible models, but we are not that far yet. By doing more QMRA studies using the MPRM methodology, our 'library of models' should grow larger, with more models that are practicable in QMRA.

An important idea behind the MPRM is to treat models independent from the availability of data: the models describe the transmission over the food pathway, and if data are lacking or insufficient this increases the uncertainty, but that should not prevent the risk assessor from building the model. In the case study it appeared difficult to consequently separate models from data: the choice of the model may depend much on the type of data that are available. Especially the difference between the steps 5 and 6 is therefore obscure.

In paragraph 1.2 we state that another characteristic of MPRM is the use of second order Monte Carlo modelling, that is the explicit separation of uncertainty and variability. Unfortunately we were unable to illustrate the latter in our example. As discussed in 3.6.1.2,

all probability distributions incorporated in the Monte Carlo model describe variability, either per 'batch of broccoli' or per package. "Uncertainty" in general was omitted from the analysis, because some sources of uncertainty in the exposure assessment are indeterminate themselves, so that we were not able to construct a sensible uncertainty distribution. Although this may be a general problem in complex risk assessments as the one shown here, we did not foresee this at the start of the research project. However, the nature of all probability distributions incorporated in the model are explicitly identified through the study. This is crucial in QMRA modelling.

A simplifying assumption in the exposure assessment was that the prevalence *B. cereus* in the raw broccoli and the contaminated ingredients was hundred percent. This assumption was based on expert opinion, but only valid for *B. cereus* in general. In the model it has been implemented as if one separate strain had a prevalence of one per batch, and mixtures of strains were neglected. This is not realistic. It allowed us to omit the modelling of prevalence until partitioning, and gave the opportunity to compare between different strains. In a MPRM we should however be able to deal with prevalences lower than one and mixtures of strains. That may be examined in future QMRA studies.

As a conclusion, this report has shown that MPRM is a promising risk assessment modelling structure. In the future it will be applied for an exposure assessment of *E. coli* O157 in beef tartare, and *Campylobacter* in poultry (and via other transmission routes).

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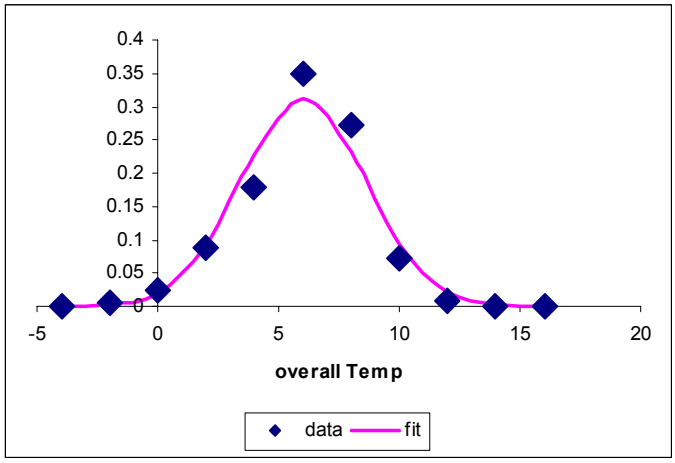
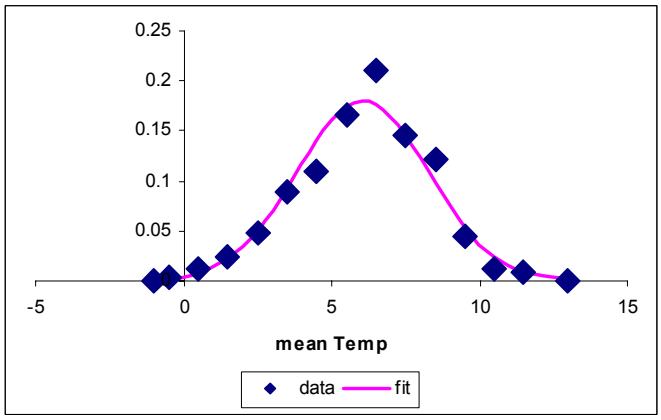
Appendix 1 Home refrigerator temperature distributions

Home fridge temperatures are variable. Not only do the temperatures vary between fridges, but also within fridges, both in time and in place in the fridge. It may be relevant to account for this variability, because, according to the square root model (see 3.4.2.3.1), the growth rate μ increases with the (sum of) square(s) of the temperature, ΣT^2. With changing temperature over time it is therefore not the mean temperature but the mean of squares of the temperature that is relevant for growth.
Evans et al. (1991) present data on temperatures of 246 consumer fridges in the UK in a study on 'Consumer handling of chilled foods'. Interestingly, they present both a distribution of mean fridge temperatures of different fridges, and an overall distribution of temperatures, both in time and per fridge. As illustrated in fig A2-1 and fig A2-2, these data fit quite well with normal distributions N(6.06, 2.21) and N(6.03, 2.56).



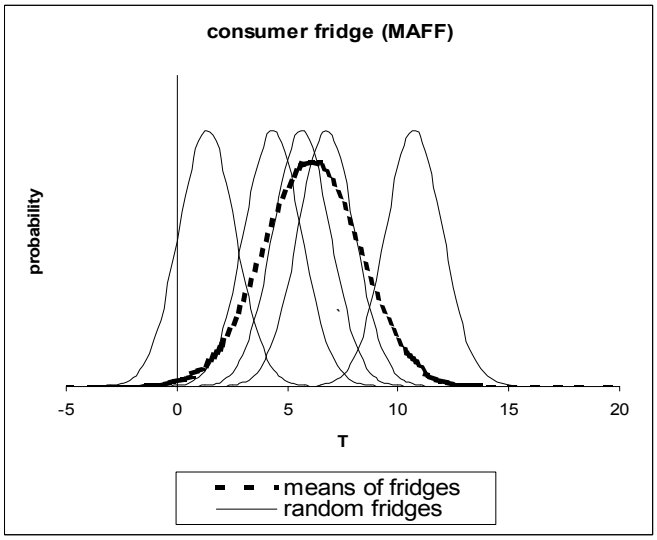
A2 1 The distribution of all temperatures measured by Evans et al. (1991) (variability in time and per fridge). Fit: N(6.03,2.56)

The means of both distributions are almost the same, so the mean fridge temperature can be set at μ =6.04. If within each fridge the temperatures vary over time according to a normal distribution with standard deviation σ, this within fridge standard deviation should have the value $\sigma = \sqrt{(2.56^2 - 2.21^2)} = 1.29$, to explain the observations of Evans et al. (1991) How the resulting 'within fridge' distributions (over time) look relative to the distribution of the means (over fridges) is illustrated for five random fridges in fig A2-3.



A2 2 The distribution of the means of temperatures measured by Evans et al. (1991) (variability per fridge). Fit: N(6.06,2.21).

The standard deviation 1.29 of temperature over time within fridges is incorporated in the exposure assessment. Assuming that t is large, for a random fridge, $E(\Sigma T^2)$ equals $m^2 + \sigma^2$, with m the mean temperature of this fridge (E(T)), a random sample from N(6.04, 2.21). As the growth rate is approximately linearly related to $E(\Sigma T^2)$, the distribution of the growth rates over the fridges can be assumed to be linearly related to $N(6.04,2.21)^2+1.29^2$.



A2-3 The distribution of the means of the fridge temperatures N(6.04,2.21) (dashed) compared to the temperature distribution with five random fridges. The mean temperature m of a random fridge is a random sample this dashed distribution. The standard deviation of the temperature within a fridge over time is 1.29.

Appendix 2 Output sheets

Below, the output sheets are given of the eleven strain/domestic fridge combinations presented in Table 3-17. The layout of these sheets is similar to the output sheet for a single iteration, as given in Fig 3-12. Additionally, this sheet shows the standard deviation in the results of 2000 iterations, next to the mean, in the right column. The (conditional) probabilities given are the means of the 2000 iterations.
The graphs in the output sheet show,
left: for the three criteria (SBD>0, tTT>1000, tTT>2000), the probability of occurrence (P(crit)), the probability of a 'risky' package, given the criterium is met (P(r|crit)), the probability of reaching the MPD, given the criterium is met (P(m|crit)), the probability of a risky package given the criterium is not met (P(r|non crit)) and the probability of reaching the MPD given the criterium is not met (P(m|non crit)).
right: the mean percentage of packages in which a 'risky' level of *B. cereus* is found; in which the MPD is reached; in which *B. cereus* has grown; that contains no cfu.; that contains spores.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome 2000 iterations avtz415europe profile.				avtz415	europe			2.1 11 juli					
2				sd										
3	log N cfu/g	before storage	-2.39	0.669079										
4	log N cfu/g	out fridge	-0.86	0.9627097					P(c)	0.68				
5		sd log N out	2.32	0.9190999										
6		risky packs	1.56%	2.09E-02		P(r)			P(r | c)	0.0210				
7		>MPD	1.91%	2.27E-02		P(>MPD)			P(c) * P(r)	0.0107				
8		past. effect	0.40	3.41E-02						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	30.39%	0.237375					P(r|crit, c)	0.09	0.04	0.24		
10		sterile packs	31.70%	0.32		1-P(c)			P(m|crit,c)	0.17	0.88	0.33		
11	mean log(# spores)per pos pack		0.05	0.08215694					P(r|non crit,c)	0.02	0.02	0.01		
12		% packs with spores	0.16	0.1542982						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.4%	5.3%		
14	# packs	overall	5.186	0.4445	5.3145	P(crit|c)			P(r|crit)	6.1%	2.5%	16.5%		
15		risky packs	0.4655	0.016	1.2815	P(r and crit |c))			P(m|crit)	11.6%	60.0%	22.6%		
16		>MPD	0.879	0.3905	1.7585	P(m and crit | c)			P(r|non crit)	1.2%	1.4%	0.6%		
17		safe	3.8415	0.038	2.2745	P(s and crit |c)			P(m|non crit)	1.4%	1.6%	0.7%		
18		risky < 0	1.635	2.0845	0.819	P(r and non crit | c)			P(r|nm,crit)	6.9%	6.1%	21.3%		
19			growth						P(r|nm,non cr)	1.2%	1.5%	0.6%		
20		Tmin max	3.30209	1.267599										
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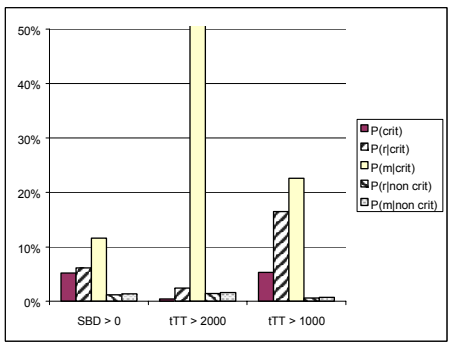

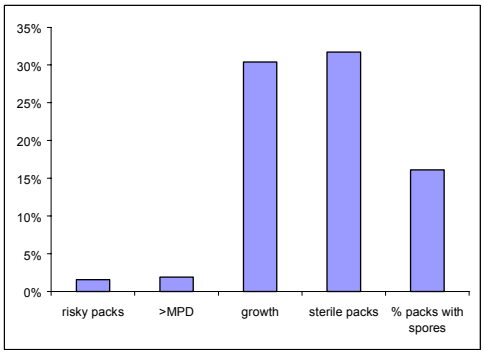

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome 2000 iterations avtz415fixed profile.				avtz415	fixed			2.1 11 juli					
2				sd										
3	log N cfu/g	before storage	-2.39	0.6682529										
4	log N cfu/g	out fridge	-1.03	1.023693					P(c)	0.68				
5		sd log N out	1.73	0.8318169										
6		risky packs	1.18%	2.06E-02		P(r)			P(r | c)	0.0159				
7		>MPD	1.03%	2.02E-02		P(>MPD)			P(c) * P(r)	0.0081				
8		past. effect	0.40	3.45E-02						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	30.70%	0.261771					P(r|crit, c)	0.11	0.05	0.22		
10		sterile packs	31.69%	0.32		1-P(c)			P(m|crit,c)	0.11	0.73	0.24		
11	mean log(# spores)per pos pack		0.05	0.08216972					P(r|non crit,c)	0.01	0.02	0.01		
12		% packs with spores	0.16	0.1568843						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.1%	3.5%		
14	# packs	overall	5.193	0.06	3.5225	P(crit|c)			P(r|crit)	7.4%	3.4%	14.9%		
15		risky packs	0.561	0.003	0.766	P(r and crit |c))			P(m|crit)	7.2%	50.1%	16.1%		
16		>MPD	0.55	0.044	0.831	P(m and crit | c)			P(r|non crit)	0.7%	1.1%	0.6%		
17		safe	4.082	0.013	1.9255	P(s and crit |c)			P(m|non crit)	0.7%	1.0%	0.5%		
18		risky < 0	1.0255	1.5835	0.8205	P(r and non crit | c)			P(r|nm,crit)	8.0%	6.8%	17.7%		
19			growth						P(r|nm,non cr)	0.7%	1.1%	0.6%		
20		Tmin max	3.315339	1.287944										
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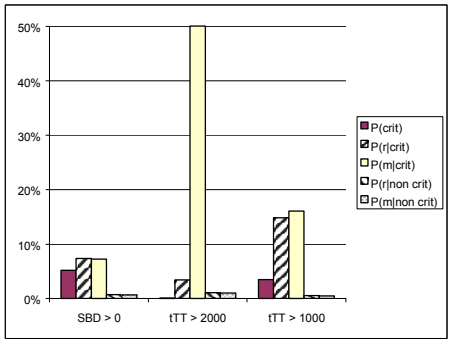

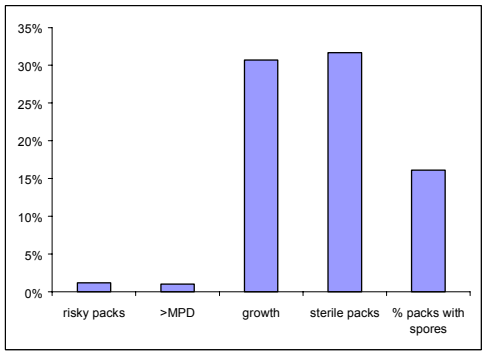

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome 2000 iterations z4222Europe profile.				avtz415	standard								
2				sd										
3	log N cfu/g	before storage	-2.38	0.6772729										
4	log N cfu/g	out fridge	-1.51	0.7930128					P(c)	0.69				
5		sd log N out	1.29	0.6923817										
6		risky packs	0.58%	1.26E-02		P(r)			P(r | c)	0.0078				
7		>MPD	0.46%	1.06E-02		P(>MPD)			P(c) * P(r)	0.0040				
8		past. effect	0.43	3.58E-02						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	19.99%	0.2300684					P(r|crit, c)	0.03	0.06	0.22		
10		sterile packs	31.21%	0.32		1-P(c)			P(m|crit,c)	0.02	0.82	0.22		
11	mean log(# spores)per pos pack		0.05	0.08905263					P(r|non crit,c)	0.01	0.01	0.00		
12		% packs with spores	0.17	0.1586425						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.0%	1.7%		
14	# packs	overall	5.1555	0.017	1.7285	P(crit|c)			P(r|crit)	2.1%	4.0%	15.2%		
15		risky packs	0.1585	0.001	0.3825	P(r and crit |c))			P(m|crit)	1.3%	56.7%	15.2%		
16		>MPD	0.099	0.014	0.381	P(m and crit | c)			P(r|non crit)	0.5%	0.5%	0.3%		
17		safe	4.898	0.002	0.965	P(s and crit |c)			P(m|non crit)	0.4%	0.4%	0.2%		
18		risky < 0	0.624	0.7815	0.4	P(r and non crit | c)			P(r|nm,crit)	2.1%	9.3%	17.9%		
19			growth						P(r|nm,non cr)	0.5%	0.5%	0.3%		
20		Tmin max	3.387487	1.304792										
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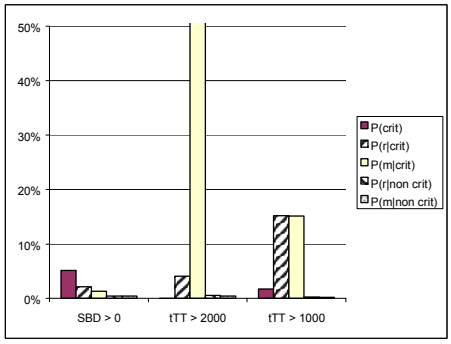

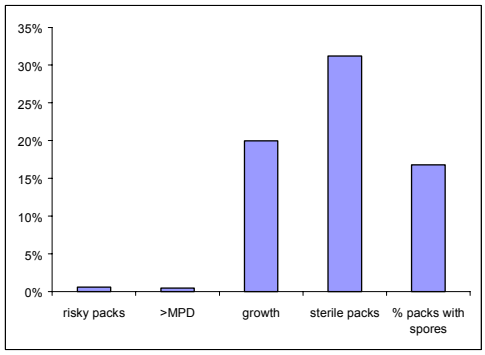

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome2000 iterations avtz415fixed profile.				avtz415	north			2.1 9 juni					
2				sd										
3	log N cfu/g	before storage	-2.39	0.6591853										
4	log N cfu/g	out fridge	-1.14	0.9065189					P(c)	0.69				
5		sd log N out	1.83	0.8150631										
6		risky packs	1.11%	1.77E-02		P(r)			P(r | c)	0.0144				
7		>MPD	1.10%	1.85E-02		P(>MPD)			P(c) * P(r)	0.0076				
8		past. effect	0.40	3.39E-02						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	27.42%	0.2383749					P(r|crit, c)	0.07	0.04	0.23		
10		sterile packs	31.33%	0.31		1-P(c)			P(m|crit,c)	0.10	0.88	0.28		
11	mean log(# spores)per pos pack		0.05	0.08309527					P(r|non crit,c)	0.01	0.01	0.01		
12		% packs with spores	16%	0.1562563						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.1%	3.4%		
14	# packs	overall	5.161	0.142	3.388	P(crit|c)			P(r|crit)	4.7%	2.9%	15.8%		
15		risky packs	0.353	0.006	0.781	P(r and crit |c))			P(m|crit)	7.0%	60.4%	19.5%		
16		>MPD	0.524	0.125	0.961	P(m and crit | c)			P(r|non crit)	0.8%	1.0%	0.5%		
17		safe	4.284	0.011	1.646	P(s and crit |c)			P(m|non crit)	0.8%	1.0%	0.5%		
18		risky < 0	1.084	1.431	0.656	P(r and non crit | c)			P(r|nm,crit)	5.0%	7.3%	19.7%		
19			growth						P(r|nm,non cr)	0.8%	1.0%	0.5%		
20		Tmin max	3.271198	1.298659										
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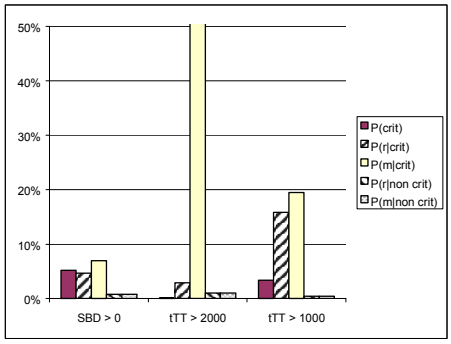

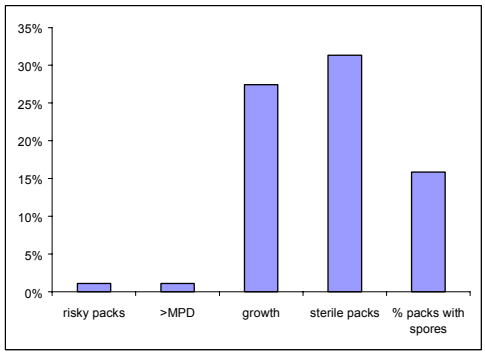

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome 2000 iterations z4222Europe profile.				avtz415	south			2.1 31 mei					
2				sd										
3	log N cfu/g	before storage	-2.37	0.6778953										
4	log N cfu/g	out fridge	-0.18	1.093305					P(c)	0.69				
5		sd log N out	3.21	1.126695										
6		risky packs	2.37%	2.55E-02		P(r)			P(r | c)	0.0324				
7		>MPD	4.03%	3.42E-02		P(>MPD)			P(c) * P(r)	0.0163				
8		past. effect	0.43	3.54E-02						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	37.86%	0.2489188					P(r|crit, c)	0.10	0.03	0.22		
10		sterile packs	30.95%	0.32		1-P(c)			P(m|crit,c)	0.32	0.89	0.39		
11	mean log(# spores)per pos pack		0.05	0.08417533					P(r|non crit,c)	0.03	0.03	0.01		
12		% packs with spores	0.17	0.1580133						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	1.3%	9.9%		
14	# packs	overall	5.1845	1.3215	9.8705	P(crit|c)			P(r|crit)	7.2%	1.8%	15.5%		
15		risky packs	0.5415	0.035	2.219	P(r and crit |c))			P(m|crit)	21.8%	61.6%	27.0%		
16		>MPD	1.6385	1.1785	3.8575	P(m and crit | c)			P(r|non crit)	2.0%	2.2%	0.8%		
17		safe	3.0045	0.108	3.794	P(s and crit |c)			P(m|non crit)	3.1%	3.3%	1.5%		
18		risky < 0	2.6945	3.201	1.017	P(r and non crit | c)			P(r|nm,crit)	9.2%	4.8%	21.3%		
19			growth						P(r|nm,non cr)	2.0%	2.3%	0.8%		
20		Tmin max	3.313239	1.283751										
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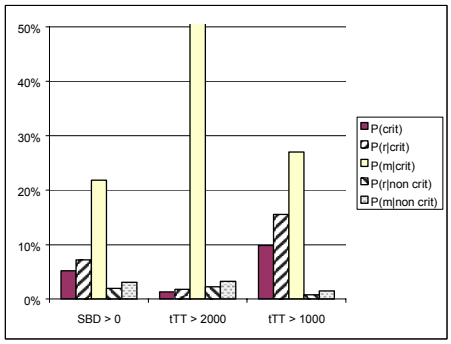

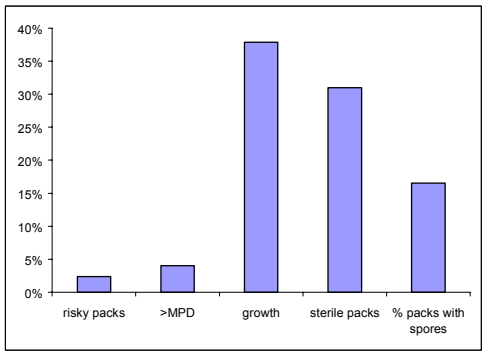

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome 2000 iterations z4222Europe profile.				z4222	europe								
2				sd										
3	log N cfu/g	before storage	-2.53	0.675198										
4	log N cfu/g	out fridge	-1.74	0.5872862					P(c)	0.62				
5		sd log N out	1.23	0.6598739										
6		risky packs	0.33%	7.17E-03		P(r)			P(r | c)	0.0048				
7		>MPD	0.36%	7.30E-03		P(>MPD)			P(c) * P(r)	0.0020				
8		past. effect	0.30	2.98E-02						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	14.67%	0.1695667					P(r|crit, c)	0.04	0.22	0.08		
10		sterile packs	37.64%	0.33		1-P(c)			P(m|crit,c)	0.05	0.49	0.06		
11	mean log(# spores)per pos pack		0.05	0.09419672					P(r|non crit,c)	0.00	0.00	0.00		
12		% packs with spores	0.15	0.1383839						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.5%	5.8%		
14	# packs	overall	5.1855	0.4855	5.833	P(crit|c)			P(r|crit)	2.6%	13.9%	5.0%		
15		risky packs	0.2165	0.1085	0.4705	P(r and crit |c))			P(m|crit)	3.3%	30.7%	3.9%		
16		>MPD	0.271	0.239	0.3605	P(m and crit | c)			P(r|non crit)	0.2%	0.2%	0.0%		
17		safe	4.698	0.138	5.002	P(s and crit |c)			P(m|non crit)	0.2%	0.2%	0.1%		
18		risky < 0	0.2655	0.3735	0.0115	P(r and non crit | c)			P(r|nm,crit)	2.7%	20.1%	5.2%		
19			growth						P(r|nm,non cr)	0.2%	0.2%	0.0%		
20		Tmin max	4.774874	1.677593										
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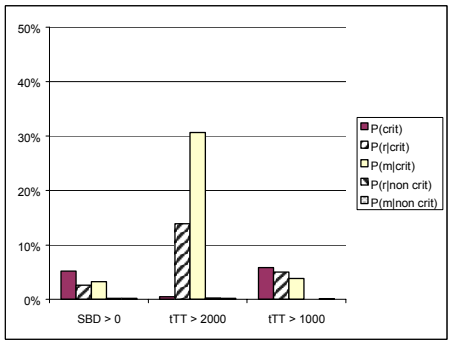

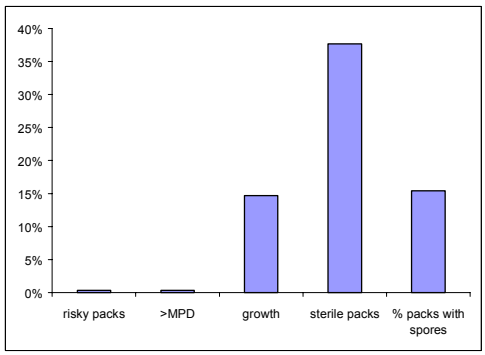

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome 2000 iterations z4222Europe profile.				avz421	europe								
2				sd										
3	log N cfu/g	before storage	-0.04	0.6777913										
4	log N cfu/g	out fridge	-0.03	0.6810279					P(c)	1.00				
5		sd log N out	0.16	0.2055791										
6		risky packs	0.03%	1.81E-03		P(r)			P(r | c)	0.0003				
7		>MPD	0.02%	1.24E-03		P(>MPD)			P(c) * P(r)	0.0003				
8		past. effect	0.92	8.50E-03						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	4.42%	0.0358295					P(r|crit, c)	0.00	0.04	0.01		
10		sterile packs	0.00%	0.00		1-P(c)			P(m|crit,c)	0.00	0.03	0.00		
11	mean log(# spores)per pos pack		1.40	0.5817651					P(r|non crit,c)	0.00	0.00	0.00		
12		% packs with spores	0.92	0.1134836						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.5%	5.9%		
14	# packs	overall	5.1915	0.457	5.883	P(crit|c)			P(r|crit)	0.2%	4.4%	0.5%		
15		risky packs	0.011	0.02	0.0315	P(r and crit |c))			P(m|crit)	0.2%	2.6%	0.2%		
16		>MPD	0.0105	0.012	0.0145	P(m and crit | c)			P(r|non crit)	0.0%	0.0%	0.0%		
17		safe	5.17	0.425	5.837	P(s and crit |c)			P(m|non crit)	0.0%	0.0%	0.0%		
18		risky < 0	0.022	0.013	0.0015	P(r and non crit | c)			P(r|nm,crit)	0.2%	4.5%	0.5%		
19			growth						P(r|nm,non cr)	0.0%	0.0%	0.0%		
20		Tmin max	9.284913	0.4991933										
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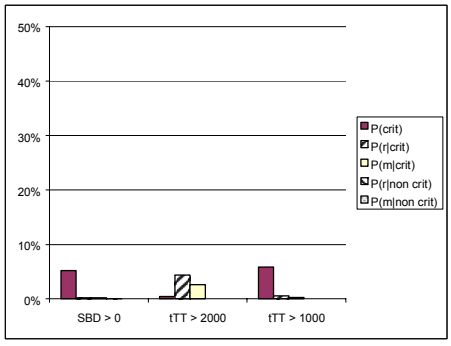

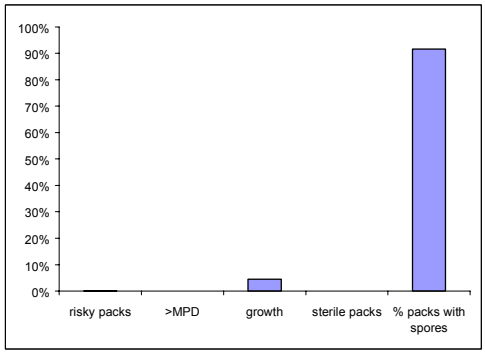

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome2000 iterations avtz415fixed profile.				AVZ421	FIXED			2.1 9 juni					
2				sd										
3	log N cfu/g	before storage	-0.03	0.6730903										
4	log N cfu/g	out fridge	-0.03	0.6754494					P(c)	1.00				
5		sd log N out	0.04	0.04782371										
6		risky packs	0.00%	0.00E+00		P(r)			P(r | c)	0.0000				
7		>MPD	0.00%	0.00E+00		P(>MPD)			P(c) * P(r)	0.0000				
8		past. effect	0.91	8.95E-03						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	2.18%	0.02681487					P(r|crit, c)	0.00	0.00	0.00		
10		sterile packs	0.00%	0.00		1-P(c)			P(m|crit,c)	0.00	0.00	0.00		
11	mean log(# spores)per pos pack		1.13	0.5716091					P(r|non crit,c)	0.00	0.00	0.00		
12		% packs with spores	90%	0.1375983						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.1%	3.6%		
14	# packs	overall	5.185	0.06	3.612	P(crit|c)			P(r|crit)	0.0%	0.0%	0.0%		
15		risky packs	0	0	0	P(r and crit |c))			P(m|crit)	0.0%	0.0%	0.0%		
16		>MPD	0	0	0	P(m and crit | c)			P(r|non crit)	0.0%	0.0%	0.0%		
17		safe	5.185	0.06	3.612	P(s and crit |c)			P(m|non crit)	0.0%	0.0%	0.0%		
18		risky < 0	0	0	0	P(r and non crit | c)			P(r|nm,crit)	0.0%	0.0%	0.0%		
19			growth						P(r|nm,non cr)	0.0%	0.0%	0.0%		
20		Tmin max	9.268068	0.498982										
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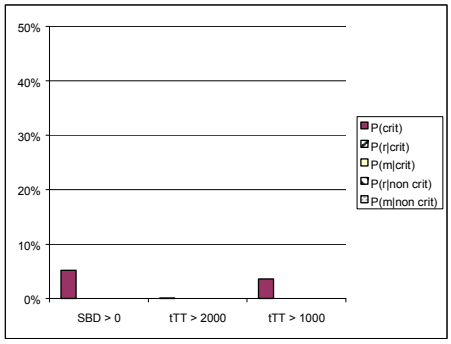

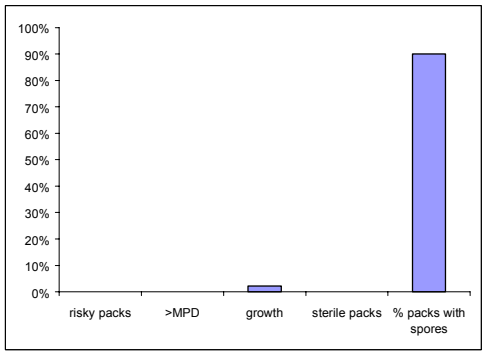

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome 2000 iterations z4222Europe profile.				l2104	europe								
2				sd										
3	log N cfu/g	before storage	-0.03	0.6863735										
4	log N cfu/g	out fridge	0.13	0.7608948					P(c)	1.00				
5		sd log N out	0.54	0.6412954										
6		risky packs	0.34%	9.69E-03		P(r)			P(r | c)	0.0034				
7		>MPD	0.31%	1.20E-02		P(>MPD)			P(c) * P(r)	0.0034				
8		past. effect	0.96	3.47E-03						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	17.22%	0.2146237					P(r|crit, c)	0.02	0.12	0.04		
10		sterile packs	0.00%	0.00		1-P(c)			P(m|crit,c)	0.03	0.29	0.04		
11	mean log(# spores)per pos pack		1.32	0.6435267					P(r|non crit,c)	0.00	0.00	0.00		
12		% packs with spores	0.91	0.1258633						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.5%	5.9%		
14	# packs	overall	5.184	0.5005	5.881	P(crit|c)			P(r|crit)	1.8%	11.9%	4.5%		
15		risky packs	0.093	0.0595	0.2635	P(r and crit |c))			P(m|crit)	2.6%	28.8%	4.4%		
16		>MPD	0.1335	0.144	0.259	P(m and crit | c)			P(r|non crit)	0.3%	0.3%	0.1%		
17		safe	4.9575	0.297	5.3585	P(s and crit |c)			P(m|non crit)	0.2%	0.2%	0.1%		
18		risky < 0	0.2435	0.277	0.073	P(r and non crit | c)			P(r|nm,crit)	1.8%	16.7%	4.7%		
19			growth						P(r|nm,non cr)	0.3%	0.3%	0.1%		
20		Tmin max	7.752028	1.992921										
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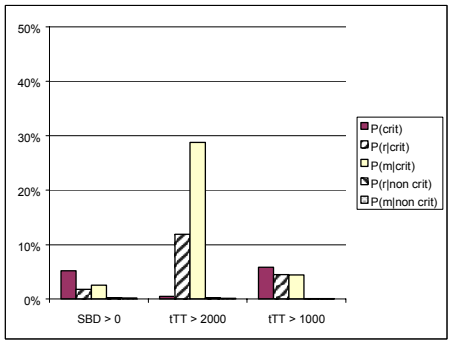

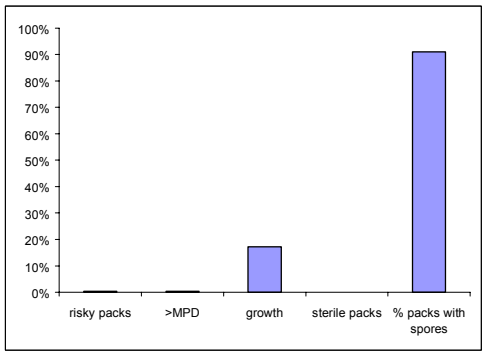

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome2000 iterations avtz415fixed profile.				l2104	FIXED			2.1 9 juni					
2				sd										
3	log N cfu/g	before storage	-0.02	0.6762694										
4	log N cfu/g	out fridge	0.06	0.7156994					P(c)	1.00				
5		sd log N out	0.19	0.3292846										
6		risky packs	0.11%	7.76E-03		P(r)			P(r | c)	0.0011				
7		>MPD	0.06%	6.20E-03		P(>MPD)			P(c) * P(r)	0.0011				
8		past. effect	0.95	3.46E-03						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	15.56%	0.2522134					P(r|crit, c)	0.00	0.06	0.02		
10		sterile packs	0.00%	0.00		1-P(c)			P(m|crit,c)	0.00	0.06	0.01		
11	mean log(# spores)per pos pack		1.16	0.594757					P(r|non crit,c)	0.00	0.00	0.00		
12		% packs with spores	90%	0.1376132						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.1%	3.5%		
14	# packs	overall	5.187	0.064	3.528	P(crit|c)			P(r|crit)	0.4%	6.3%	1.6%		
15		risky packs	0.019	0.004	0.057	P(r and crit |c))			P(m|crit)	0.2%	6.3%	0.9%		
16		>MPD	0.008	0.004	0.03	P(m and crit | c)			P(r|non crit)	0.1%	0.1%	0.1%		
17		safe	5.16	0.056	3.441	P(s and crit |c)			P(m|non crit)	0.1%	0.1%	0.0%		
18		risky < 0	0.091	0.106	0.053	P(r and non crit | c)			P(r|nm,crit)	0.4%	6.7%	1.6%		
19			growth						P(r|nm,non cr)	0.1%	0.1%	0.1%		
20		Tmin max	7.720942	2.058323										
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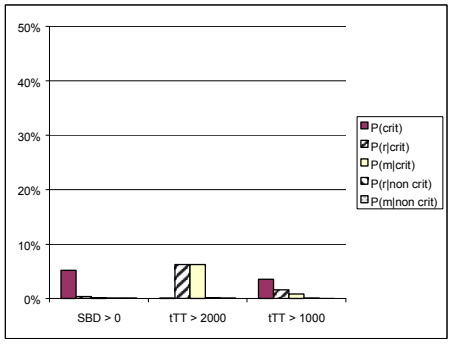

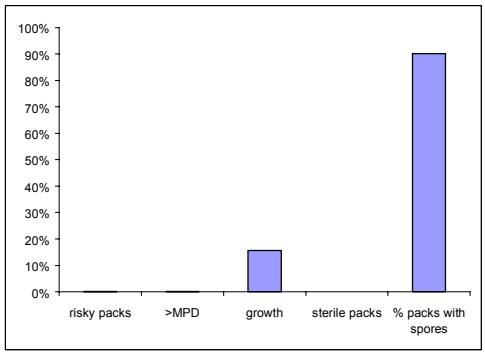

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Mean outcome 2000 iterations z4222Europe profile.				z4222	europe								
2				sd										
3	log N cfu/g	before storage	-0.05	0.6829104										
4	log N cfu/g	out fridge	0.02	0.6990349					P(c)	1.00				
5		sd log N out	0.36	0.5180528										
6		risky packs	0.15%	4.78E-03		P(r)			P(r | c)	0.0015				
7		>MPD	0.16%	4.93E-03		P(>MPD)			P(c) * P(r)	0.0015				
8		past. effect	0.92	8.47E-03						SBD > 0	tTT > 2000	tTT > 1000		
9		growth	7.93%	0.09075148					P(r|crit, c)	0.01	0.07	0.02		
10		sterile packs	0.00%	0.00		1-P(c)			P(m|crit,c)	0.01	0.17	0.02		
11	mean log(# spores)per pos pack		1.33	0.6102381					P(r|non crit,c)	0.00	0.00	0.00		
12		% packs with spores	0.91	0.1252761						SBD > 0	tTT > 2000	tTT > 1000		
13			SBD > 0	tTT > 2000	tTT > 1000				P(crit)	5.2%	0.5%	5.8%		
14	# packs	overall	5.1895	0.474	5.849	P(crit|c)			P(r|crit)	0.9%	7.1%	2.1%		
15		risky packs	0.0465	0.0335	0.1215	P(r and crit |c))			P(m|crit)	1.5%	17.0%	2.4%		
16		>MPD	0.076	0.0805	0.14	P(m and crit | c)			P(r|non crit)	0.1%	0.1%	0.0%		
17		safe	5.067	0.36	5.5875	P(s and crit |c)			P(m|non crit)	0.1%	0.1%	0.0%		
18		risky < 0	0.102	0.115	0.027	P(r and non crit | c)			P(r|nm,crit)	0.9%	8.5%	2.1%		
19			growth						P(r|nm,non cr)	0.1%	0.1%	0.0%		
20		Tmin max	9.231964	1.840868										
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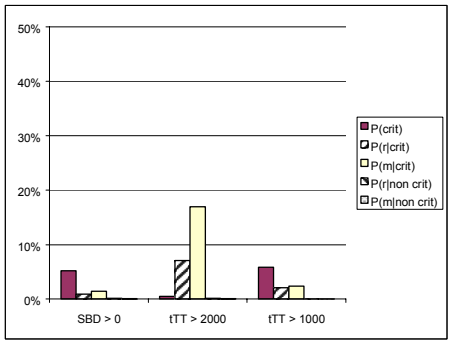

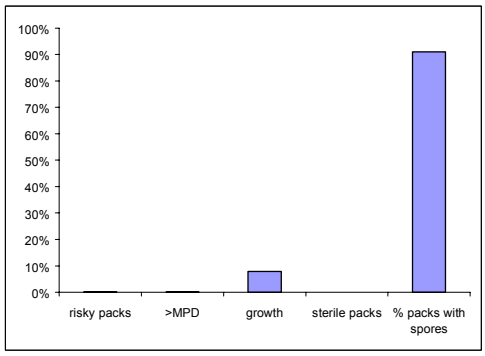

Appendix 3 Mailing list

1. Plv. Directeur-Generaal Volksgezondheid, VWS, Drs. N.C. Oudendijk
2. Algemeen directeur, Keuringsdienst van Waren, VWS, Dr. ir. M.W.J. Wolfs
3. Hoofd Accountsectie Food, Keuringsdienst van Waren, VWS, Prof. dr. P. Peters
4. Keuringsdienst van Waren, VWS, Drs. J.T. Jansen
5. Keuringsdienst van Waren, VWS, Ir. J. van Kooij
6. Keuringsdienst van Waren, Regio Zuid, Dr. P. in 't Veld
7. Keuringsdienst van Waren, regio Noord-West, Drs. F. de Jonge
8. Directie Gezondheidsbeleid, Drs. A.A.W. Kalis
9. Voorzitter van de Gezondheidsraad, Prof. J.J. Sixma
10. Dr. R. Beumer, Wageningen Universiteit en Research Centrum, Wageningen
11. Dr. F. Carlin, Istitut National de la Recherche Agronomique, Avignon, France
12. Dr. M. Peck, Institute of Food Research, Norwich, UK
13. Dr. G. Barker, IFR Norwich UK
14. Dr. P. Malakar, IFR Norwich UK
15. Dr. A. Martinez, Instituto de Agroquemica y Technologia de Alimentos, Valencia, Spain
16. Dr. P. Fernandez, Universidad Politechnica de Cartagena, Cartagena, Spain
17. Dr. W. Waites, University of Nottingham, UK
18. Dr. R. Moezelaar, ATO-DLO, Wageningen
19. Dr. M. Del Torre, Uinversity di Udin, Facolta de Agraria, Udine, Italy
20. Mrs. S. Litman, Syndicat National des Fabricants de Plats Cuisinés, Paris, France
21. RANA, Italy
22. Alvalle, Spain
23. Ewen Todd, Bureau of Microbial Hazards Canada
24. Anna Lammerding, Microbial Food Safety Risk Assessment Canada
25. Don Schaffner, University of New Jersey
26. Peg Coleman, U.S. Department Agriculture
27. Tanya Soboleva, Agriculture Research Institute New Zealand
28. Suzanne van Gerwen, Unilever research Vlaardingen
29. Servé Notermans, TNO Voeding Zeist
30. Taco Wijtzes, TNO Voeding Zeist

31. Erik Hoornstra, TNO Voeding Zeist
32. Marcel Zwietering, Danone Paris
33. Depot Nederlandse Publikaties en Nederlandse Bibliografie
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35. Prof. dr. ir. D. Kromhout, Directeur sector 2
36. Dr. ir. A.M. Henken, Hoofd MGB
37. Dr. ir. A. Havelaar, Plv. hoofd MGB
38. F.M. van Leusden, Projectleider MGB
39. E.G. Evers, MGB
40. J.F. Schijven, MGB
41. K. Takumi, MGB
42. R. de Jonge, MGB
43. L.M. Wijnands, MGB
44. J.B. Dufrenne, MGB
45. W. Slob, LEO
46. P.F.M. Teunis, IMA
47. Auteur(s)
48. SBD/Voorlichting & Public Relations
49. Bureau Rapportenregistratie
50. Bibliotheek RIVM

51-65 Bureau Rapportenbeheer

66-70 Reserve-exemplaren