Microbiological Risk Assessment of Food

A stepwise quantitative risk assessment as a tool in the production of microbiologically safe food

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Stellingen

1. Het ontbreken van kwantitatieve data belemmert een risicobepaling niet noodzakelijkerwijs, sterker nog: het kan leiden tot duidelijke inzichten in de risicobepalende factoren.

2. Zonder helderheid in de risicobepaling is voor risicomanagement een willekeurige schatting een sneller en niet minder waardevol alternatief.

3. Vrijwel alle gepubliceerde voorbeelden van microbiologische 'kwantitatieve risicobepaling' voor levensmiddelen zijn feitelijk geen risicobepaling, omdat het fenomeen 'ernst van de gevolgen' buiten beschouwing wordt gelaten.

4. De voorspellende microbiologie is niet of nauwelijks gebaat bij artikelen over empirische groeimodellen als niet de gefitte parameters, maar slechts de voorspellingen van het model weergegeven worden.

   zie o.a. Sutherland & Bayliss, 1994, Int.J.Food Microbiol., 21, 197-215; Sutherland et al., *1996, Int.J.Food Microbiol., 30, 359-372.*

5. Als een methode slechts aan de hand van een voorbeeld uitgelegd kan worden, is de methode niet goed gedefinieerd.

   Zie bijvoorbeeld: Marks et al., *1998, Risk Analysis, 18, 309-328*

6. Volgens de filosoof Popper is de waarheid van een theorie niet te bewijzen, maar slechts aannemelijk te maken door te streven naar falsificatie. De praktijk laat zien dat velen er anders over denken.

   Popper,K.R., 1969, Conjectures and Refutations. The growth of scientific knowledge, London, Routledge and Kegan Paul.
   Voor hitteinactivatie van sporen zie: Casolari, 1994, Food Microbiol., 11, 75-84.

7. Door het beschikbaar komen van steeds betere rekenprogramma's is de kans groot dat men de essentie van een probleem over het hoofd ziet.

8. Eenvoud is niet eenvoudig.

9. Daar vele mannen in de zomer zonder schroom hun bovenlichaam ontbloten, is het niet verwonderlijk dat nog steeds de vrouw in plaats van de man als lustobject gezien wordt.

10. Het vermogen van de mens om zich aan te passen aan moeilijke omstandigheden is een zegen voor het dagelijks leven, maar blijkt op lange termijn veelal een zoethouder te zijn.

    N.a.v. De Tocqueville, 1839 'Should I call it a blessing of God, or a last malediction of his anger, this disposition of the soul that makes men insensible to extreme misery ?'. Geciteerd door: Murray, C.J.L, 1996, Ch. 1. In: The Global Burden of Disease, WHO.

11. Voor exotische vakantiebestemmingen slikt men het middel Lariam tegen malaria, met als mogelijk bijverschijnsel depressiviteit. Dat is toch zonde van je vakantie.

12. Bij de huidige trend de overheid verantwoordelijk te stellen voor allerhande onverwachte gebeurtenissen is het paradoxaal dat haar eveneens verweten wordt zich overal mee te bemoeien.

13. Een dropping in een weiland in de polder zou tot grote verrassingen leiden voor mensen die alsmaar klagen dat Nederland te vol is.

Stellingen behorende bij het proefschrift 'Microbiological Risk Assessment of Food'.

Suzanne van Gerwen
Wageningen, 14 februari 2000

Microbiological Risk Assessment of Food

A stepwise quantitative risk assessment as a tool in the production of microbiologically safe food

Microbiologische Risicobepaling van Levensmiddelen
Stapsgewijze kwantitatieve risicobepaling als instrument bij de productie van microbieel veilige levensmiddelen

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Abstract

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Key words: microbiological food safety, quantitative risk assessment, hazard identification, predictive models, inactivation, expert system.

In this thesis a method for quantitative microbiological risk assessment is presented. An expert system has been developed to assess risks, and find risk-determining phenomena, for relevant microbial hazards related to foods and food production processes in general. As such, it is a useful tool in HACCP studies. The expert system has implemented literature and expert knowledge as databases, and combines these databases to microbial predictive models.

The method for quantitative risk assessment (QRA) has been named the SIEFE model: Stepwise and Interactive Evaluation of Food safety by an Expert system. The stepwise approach consists of starting simple before going into detail, to obtain clear insight into the risk assessment process.

The SIEFE model's first step is hazard identification. This thesis describes a structured, interactive procedure to select relevant hazards for food products.

Another aspect of QRA that has been described in this thesis is the use of predictive models in stepwise QRA. Simple models were shown to be useful, even in detailed risk assessments.

The thesis also describes the results of a data analysis of the irradiation parameter D_{10}. The data analysis has resulted in a categorisation of D_{10}, related to quantitatively relevant factors. The categorisation helps to predict the effectiveness of any irradiation process, and is a useful guide in designing safe food processes.

The SIEFE model's stepwise approach highlights quantitatively relevant phenomena, and allows omission of non-relevant aspects based on explicit reasoning. This gives the best insight into the complex field of risk assessment, and prevents the user from getting caught in too much details. The stepwise approach provides transparancy in risk assessments, which is a must for good decision-making in this area.

Contents

1

Introduction: Quantitative risk assessment as a tool in the production of safe foods

1. Quantitative risk assessment - theory

Food safety is not a subject which frequently occurs in commercials (16). Consumers have confidence in buying safe foods, so no direct profits can be gained from selling a safe product. Harming this confidence, such as in the recent dioxin affair with chicken in Belgium (June 1999), generally leads to important negative economic consequences for food producers. Next to the costs of recalls, damage of a company's good reputation affects sales. It may take years for a food producer to fix the damage of reputation. Think of Austrian wine, and many people will recall the problems with anti-freezing agents about 15 years ago.

Shortly, food producers cannot gain direct profits from controlling food safety, instead they have much to lose if their products turn out to be unsafe.

Consequently, food industry has taken much interest in food safety in the past years. Research centres, regulatory agencies, and food related companies have put large efforts in developing food safety management systems, and a new market of food safety management services and products has been developed. The Hazard Analysis Critical Control Point (HACCP) system is a food safety management system that is widely applied for systematically controlling food production processes. In the European Community, it is mandatory for food producers to apply the HACCP principles (5).

Many of the contemporary food safety management systems in food companies are used mainly qualitatively. A quantitative approach of food safety is however beneficial compared to a qualitative approach, since it gives quantitative insight into production processes, and can estimate consequences of purposeful, or unexpected and uncontrollable changes in process parameters. This enables efficient evaluation of food production processes. A quantitative approach of food safety management systems can be developed by quantitative risk assessment (QRA).

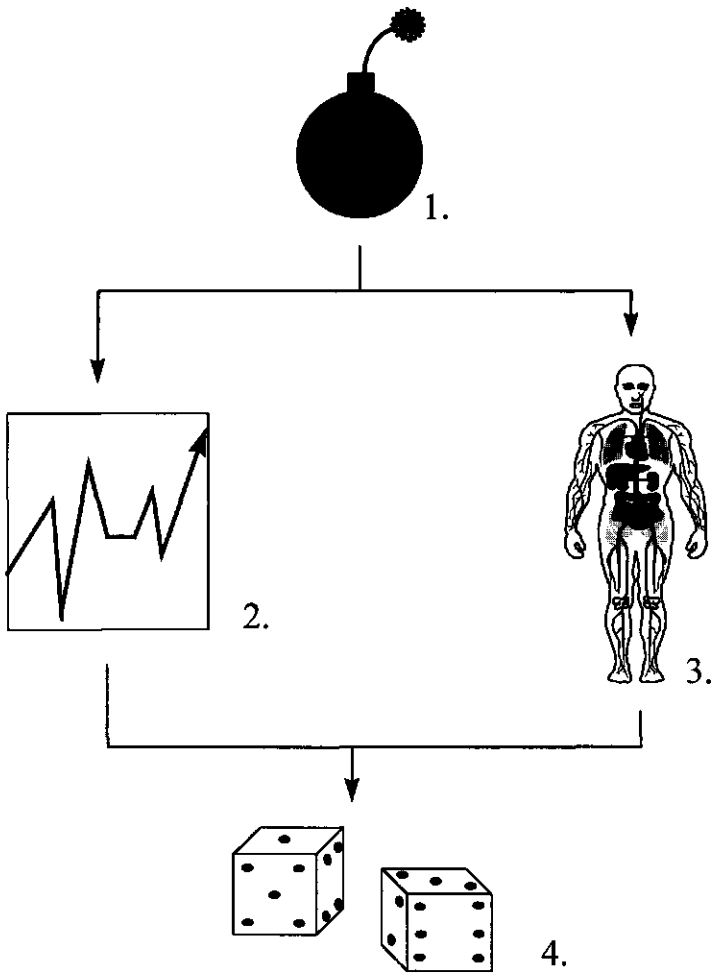

Fig. 1. Schematic representation of quantitative risk assessment: 1. hazard identification; 2. exposure assessment; 3. hazard characterisation; and 4. risk characterisation.

By estimation of changes in the concentration of a hazard per process step, QRA helps finding critical process steps for food safety. As regards this, QRA can be a useful supplement to HACCP studies. QRA also enables easy comparison of various hazards, resulting in hazard and risk ranking. Hazard and risk ranking facilitate risk management decisions. QRA is therefore an important tool for effective control of relevant food safety hazards.
Quantitative risk assessment consists of four aspects (2,8,11,12), as schematically shown in Figure 1. The four aspects are shortly explained in the following paragraphs.

1.1 Hazard identification
The CODEX definition of hazard identification is: The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods (2). A hazard has been defined as a biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect. Microbiological hazards are for example: *Salmonella enteritidis* and *Bacillus cereus*; chemical hazards are for example: carcinogens, pesticides, and anti-nutritional components; and physical hazards are for example: pieces of glass and pieces of metal.

1.2 Exposure assessment
Exposure assessment has been defined as the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant (2).
After identifying relevant hazards for a food product, it is important to estimate the hazards' fate until consumption of the product. For exposure assessment it is for example relevant to know a hazard's probability of presence, the level of contamination, and changes in the concentration of the hazard. Quantitative description of the behaviour of a hazard provides an estimate of the amount of the hazard present in a food product at the time of consumption. It is this amount that is called the exposure of the consumer to the hazard.

1.3 Hazard characterisation
If a hazard enters a person by food consumption, the person may get health-problems depending on the amount of the hazard consumed. Hazard characterisation is the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in

food (2). Dose response assessment is the determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response) (2). Dose response data may for example express a threshold value. Before the threshold value the probability of health problems is zero, and after the threshold value this probability is one. For infectious microorganisms, dose response data are often described by parameters of a sigmoide curve, relating the logarithmic amount of organisms to the probability of infection.

1.4 Risk characterisation

Coupling exposure to dose response data results in an estimate of the risk of having health problems related to consumption of a certain product. Risk characterisation has been defined as: the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment (2). In many cases it is impossible to accurately estimate risk, due to large uncertainties and inaccuracies in exposure and dose response assessment. Even if risk cannot be estimated accurately, risk and hazard ranking are often useful and supportive for decision making. Especially if the risk assessment has been transparent, and the steps in risk assessment can be evaluated critically, order of magnitude estimates of risk can be a useful tool in properly allocating resources.

2. Quantitative risk assessment - practice

The problem of uncertainties and inaccuracies in QRA will be very difficult to address if accurate estimation of risk is the only objective of QRA. It is a fact that many aspects related to QRA have not been described quantitatively. Moreover, the majority of aspects that have been estimated or modelled quantitatively, can only be estimated as order of magnitudes. For bacteria for example, a large amount of growth models have been developed in past years. Many models have been validated in food products, but deviations up to a factor 10 or more between predicted and measured growth rates, generation times or doubling times were shown regularly (3,7,10,14,15,17).

Even if only microbiological hazards are considered, many aspects can be listed that were not or hardly described quantitatively. Some of these are listed below.

- The prevalence and size of contamination of ingredients with selected hazards are often unknown in practice, since most inspections on ingredients only occur for several (groups of) microorganisms, for example, the total aerobic plate count, coliforms, and *Escherichia coli.* Prevalence of contamination is very difficult to measure anyway, especially for low prevalence; imagine the amount of samples necessary to prove that one in a thousand products is contaminated. Henzler *et al.* (6) selected a sample size of 3000 with the intention of detecting *Salmonella* positive eggs at a prevalence of 1/1000 with 95% confidence (assuming a fully effective assay). Given this, imagine the experimental validation of a 12D reduction of *Clostridium botulinum.*
- Various growth models are available for estimation of growth. There is however no certainty on the model that will best predict growth in the specific situation studied, so it is not sensible to rely on one model only. Moreover, the parameters of many models (especially response surface models) are often unknown for the situation studied. The same applies to inactivation models.
- Toxin formation is directly or indirectly related to growth of toxinogenic pathogens. For several toxinogenic pathogens, there are general rules on the increase allowed without food-poisoning problems to occur. *Staphylococcus aureus* growth for example, is generally known to cause no problems up to 10^4 CFU·g^{-1}. Various quantitative models describing (the probability of) toxin formation have been published, and the vast majority of these models is for *Clostridium botulinum* (1,4). For other toxinogenic pathogens, there is little quantitative insight into conditions leading to formation of dangerous amounts of toxin. At present, one depends on general expert knowledge on toxin formation.
- Heat treatment has been the mostly used process to inactivate microorganisms for many years. Various new techniques for food preservation are emerging, for example food irradiation, high pressure treatment, and pulsed electric fields. The importance of factors that quantitatively influence the inactivation parameters are often unknown, and therefore it is difficult to quantitatively estimate the inactivation parameters under various conditions.
- Recontamination after inactivation can be risk-determining, especially if the hazard is able to grow in the product. Recontamination can occur in many ways, for example by workers' hands, by contaminated stagnant areas, and by contaminated contact surfaces. At present, quantitative data and models to estimate recontamination are scarce (e.g. 9,13,18), so estimation of recontamination needs specific experimental results, or creative guessing.

- Dose response data are available for only a few infectious and toxico-infectious pathogens. Moreover, for those known, accuracy is often rather low, especially in the practically relevant low ranges. The large differences in virulence and infectivity of various strains make it difficult to apply the dose-response relation for one organism to another organism. Large differences in susceptibility of humans also form a problem in dose-response assessment, and it is very difficult to weigh various health effects.

The above examples show some practical problems with regard to accurate estimation of microbiological risk. It is very doubtful whether the benefits of accurate estimation of risk outweigh the difficulties. Rough quantitative insight generally provides enough information to focus on the quantitatively relevant aspects and thereby to significantly contribute to decision making.

The above examples were for microbiological hazards only. Besides microbiological and other biological hazards, the scope of QRA for food products exists of chemical and physical hazards (2). Microbiological hazards are generally alive, and may grow and die during the production process, if present. Chemical hazards may be formed, or broken down into harmless substances during the production process. In that sense they are comparable to microbiological hazards. Chemical hazards may however be formed without actual (external) contamination of the hazard, or may naturally be present in the ingredients of the product. As with microbial hazards, physical hazards are generally introduced into the product by external factors. In contrast to microbiological and chemical hazards, physical hazards do however not grow, and are not formed in the product during the production process. They cannot be inactivated, but may be removed.

Considering the large variety of hazards and their behaviour in foods, supplemented with the large variety of foods, it seems impossible to systematically assess risks for food products in general. This thesis shows that systematically assessing bacterial risks is helpful to structure the problem and to make best decisions on data available. The large variety of risk-related aspects is not considered to be a problem that overwhelms the risk assessor; instead the risk-assessor is guided in omitting non-relevant aspects, and focusing on risk-determining aspects. It is therefore expected that systematically assessing risks for any hazard in food products in general is a realistic opportunity for the future.

3. Objective of the thesis

The objective of the thesis is to describe microbial quantitative risk assessment as a tool for the production of safe foods. Regarding this, a transparent, stepwise procedure for quantitative risk assessment for food products in general is developed, and various aspects of quantitative risk assessment are studied in detail. The procedure is supportive for decision makers in food safety by giving quantitative insight into microbial behaviour during production processes. Conventional products and processes, variations to products and processes, and new products and processes can be studied to find critical steps related to food safety. The procedure is transparent, meaning that the results can be evaluated critically. The stepwise approach efficiently focuses on aspects that are truly relevant, and also detects phenomena that are not quantitatively important and do not have to be studied in further detail.

4. Outline of this thesis

The chapters 2, 3, and 4 of this thesis describe various aspects related to quantitative risk assessment, and chapters 5, 6, and 7 concern an overall procedure for microbiological quantitative risk assessment.

Chapter 2 of this thesis deals with the first part of risk assessment; hazard identification. A stepwise and interactive procedure for bacterial hazard identification has been developed to systematically identify relevant hazards for food products. Chapter 3 compares various growth and inactivation models for their practical use in stepwise quantitative risk assessments. Chapter 4 concerns inactivation of bacteria and spores by irradiation. Factors quantitatively influencing the irradiation parameter D_{10} were studied, and a classification of D_{10} has been made. The classification can be used to estimate D_{10} values under various conditions.

A stepwise and interactive procedure for microbial quantitative risk assessment for food products is described in chapter 5. Implemented as an expert system, the procedure integrates the various steps of quantitative risk assessment. It is a structured method, coupling qualitative and quantitative knowledge on hazards to predictive models, process engineering models, and databases containing quantitative data and qualitative expert and literature knowledge. The procedure was named the SIEFE model. The SIEFE model was applied to two example products in chapter 6, to test its usefulness in providing quantitative insight into microbial contamination, growth and inactivation during food production processes. Moreover, the SIEFE model is compared to an approach for microbial quantitative risk assessment from the literature in chapter 7.

Chapter 8 is the general discussion. It deals with the SIEFE model as a tool for the production of microbiologically safe food. It also tentatively evaluates the applicability of the SIEFE model for physical and chemical quantitative risk assessment, and discusses various aspects of quantitative risk assessment that need more research in the future.

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2

An identification procedure for foodborne microbial hazards

Abstract

A stepwise and interactive identification procedure for foodborne microbial hazards has been developed in which use is made of several levels of detail ranging from rough hazard identification to comprehensive hazard identification. This approach allows one to tackle the most obvious hazards first, before focusing on less obvious hazards. The interactive character of the identification procedure is based on the use of several knowledge sources. Combination of knowledge sources, expressed in the use of knowledge rules, supports the user in systematically selecting hazards which may pose a real risk to the consumer. Due to the structured method and the clear definitions of the knowledge rules, the procedure is transparent and may be changed if necessary. The hazard identification procedure has been implemented as a computer program, resulting in a decision-supporting identification system. It provides a way to efficiently assess those hazards which may cause harm if not brought under control during processing. The procedure forms a basis for quantitative risk assessment.

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1. Introduction

The HACCP (Hazard Analysis Critical Control Points) system was developed in the early 1970s. The system is used to manage the safety of food products systematically by paying special attention to those steps in the process that are essential in the production of acceptably safe foods. In the recent past, many food processing companies have introduced safety management systems based on HACCP principles. Application of the principles of HACCP has become mandatory for food companies in the European Community (12). The HACCP system is however often used qualitatively and subjectively. A quantitative approach of the HACCP system provides a better way to set proper criteria for critical process steps (indicated as CCPs), to execute control measures, and to optimise processes according to a certain risk. The quantitative approach can be created by the implementation of quantitative risk analysis in existing HACCP systems (6,9,22).

Quantitative risk analysis is based on quantitative data and models and consists of six activities: (i) hazard identification; (ii) exposure assessment; (iii) dose-response assessment; (iv) risk characterisation; (v) risk management; and (vi) risk communication. Steps 1 to 4 are termed risk assessment.

As shown in Table 1, hazard identification is the first activity in both quantitative risk analysis and HACCP. The importance of identification of hazards is mentioned in almost every reference dealing with quantitative risk analysis and HACCP. However, a systematic approach to the identification of hazards for food products is hardly described anywhere. Such an approach is deemed necessary to prevent pathogens relevant to products being disregarded and is especially necessary for newly developed and modified products, because new hazards may arise in these products. Only Notermans et al. (24) presented a general approach to the systematic identification of microbiological hazards for food products. This approach inspired the current development of a computer aided system for hazard identification. Our hazard identification procedure differs from Notermans' approach mainly by a stepwise identification of important hazards and its interactive character. Stepwise identification of relevant hazards is based on the use of three levels of detail ranging from rough hazard identification to comprehensive hazard identification. The interactive character results from systematically using several knowledge sources in identifying hazards. The knowledge sources are: literature knowledge, expert knowledge, and the user's knowledge.

1.1 Quantitative risk analysis: terms and definitions

Several definitions for terms in quantitative risk analysis can be found in the literature. For the purposes of this research, working definitions for *hazard* and *hazard identification* have been set up.

Hazard, in food production, is often defined as a substance that has the potential to cause harm (8,13). Hazard is also defined as an event, like unacceptable growth or survival of pathogens (15). In HACCP practice a combination of both definitions is often used. In describing the hazard identification procedure the first definition is used, so a hazard is considered to be a harmful substance instead of an event.

Hazard identification can be defined as the qualitative indication of potentially adverse health effects associated with exposure to foodborne agents (25,28). Notermans & Teunis (23), and Bernard & Scott (3) on the contrary, define hazard identification as a qualitative indication of the hazards that may be associated with the consumption of a particular food product. It is this latter definition that is used in this chapter.

Table 1. Steps in quantitative risk analysis and in the HACCP system

Quantitative risk analysis		***HACCP*** (8)
1. Hazard identification	Risk assessment	1. Hazard analysis: hazard identification, assessment of likelihood of occurrence of hazards and identification of preventative measures for their control.
2. Exposure assessment		2. Determine CCP's
3. Dose-response assessment		3. Establish critical limits
4. Risk characterisation		4. Establish a monitoring system
5. Risk management		5. Establish corrective actions
6. Risk communication		6. Establish verification procedures
		7. Establish documentation

2. An outline of the hazard identification procedure

The hazard identification procedure is shown in Fig. 1. The starting point of the hazard identification procedure is a list of microorganisms that are known to be pathogenic to man. Currently the list contains about 200 names of pathogens. Then three options can be selected: (i) rough hazard identification; (ii) detailed hazard identification; and (iii) comprehensive hazard identification. The process of consecutively using the levels of detail is illustrated in Fig. 2.

The reason for this approach is to perform risk assessments and control risks for the most relevant hazards before doing so for less expected hazards. The use of the levels of detail provides a way to maintain stepwise focus on the most important aspects with respect to risk assessment.

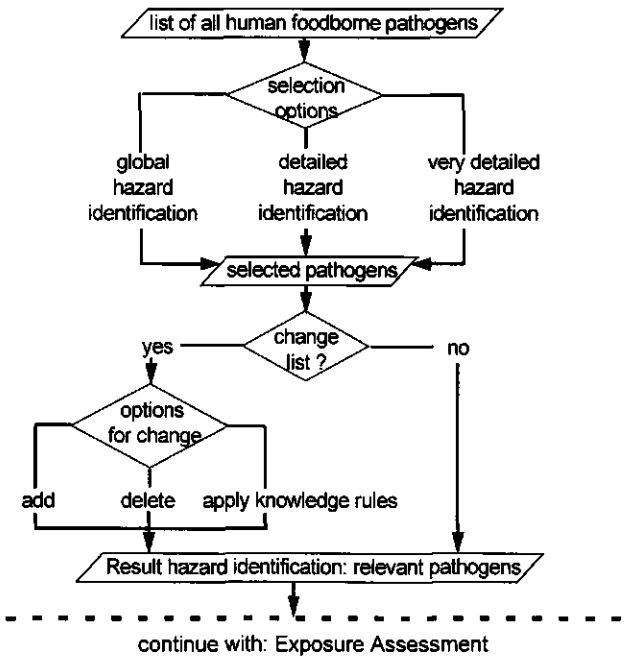

Fig. 1. Hazard identification procedure.

The detailed and comprehensive hazard identification may result in a long list of pathogens that is impractical to work with. It is efficient to start with the most relevant hazards of this list. The user can be supported in selecting these pathogens by the use of literature and expert knowledge. Literature knowledge is useful for selection of theoretically hazardous pathogens, whereas expert knowledge is useful to treat theoretical predictions with relativism. Literature and expert knowledge have been captured in knowledge rules. The user decides which knowledge rules are applied in the hazard identification. It is this combination of various knowledge sources that provides the dynamic and interactive character to the hazard identification procedure. The final result of the hazard identification procedure is a practical list of relevant pathogens. Risks can be assessed for these pathogens in the first instance.

In this chapter, the three levels of detail and the knowledge rules are described followed by the implementation of the hazard identification procedure as a decision-support system. Finally, the hazard identification procedure applied to several food products is described as an example.

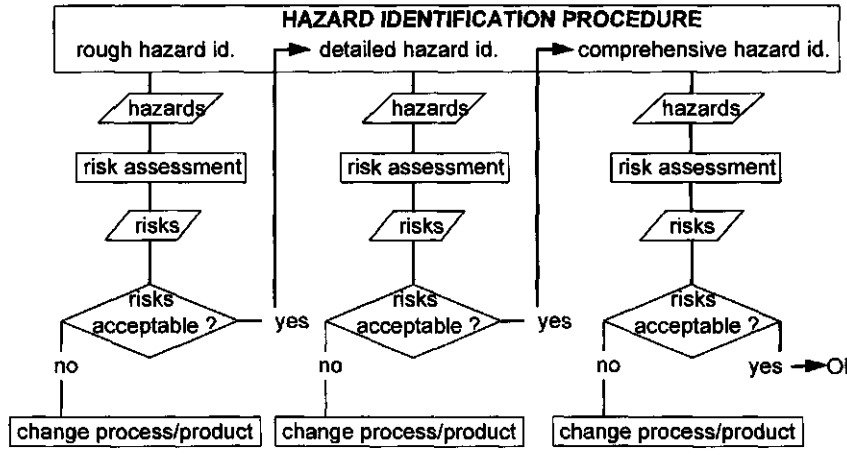

Fig. 2. Process of using several levels of detail in the hazard identification procedure.

3. Hazard identification at three levels of detail

3.1 Rough hazard identification

The rough hazard identification selects pathogens that were reported to have caused foodborne outbreaks in the selected product in the past. These pathogens are the most obvious since they have caused health problems via the specified product, whereas other pathogens did not. Much data on foodborne-outbreaks and related pathogens can be found in the literature (2,32). Only a small proportion of all foodborne illness has however been reported to the authorities (7,24) and it has often been very difficult to determine which pathogen in which food item was the true causative agent at the moment of consumption. Moreover, food products often contain a variety of ingredients that could have been the source of the causative agents, yet foodborne outbreaks are mostly listed only under the food product (2). However, if a case has been reported for a specified product it is reasonable to start a risk assessment for the causative pathogen.

3.2 Detailed hazard identification

The detailed hazard identification selects pathogens that have been reported as being present in the ingredients of the specified product. In literature many data can be found on ingredients with associated pathogens (1,17). Pathogens that have been introduced into the product by ingredients may cause health problems if the production process is not properly controlled.

3.3 Very detailed hazard identification

The comprehensive hazard identification procedure identifies all human pathogens as hazardous. By this means, pathogens that unexpectedly recontaminate the product can be included. The cases of previously unknown contamination of dried infant formula with *Enterobacter sakazakii* in 1989 (4,30) are examples of unexpected hazards. It was suspected that infant formula had been contaminated during the manufacturing process. The reservoir and mode of transmission of *Enterobacter sakazakii* has however not been clearly identified (21).

By risk assessments for unexpected hazards and unexpected events (failure analysis) it is possible to estimate the food safety consequences of the occurrence of unexpected events. In this way it is possible to get an impression of possible problems in the future and to deal with them pro-actively.

4. Knowledge rules to be used in hazard identification

Knowledge rules can be used to reduce an impractically long list of pathogens in a systematic and well-founded manner, such that the hazards that are of most likely relevance for the specific product can be assessed.

Three types of knowledge rules are used in the procedure (Table 2); 1, rules concerning presence or absence, and survival or inactivation of pathogens; 2, general rules on pathogen characteristics; 3, rules concerning growth opportunities and toxin production.

Type 1 rules select pathogens that are present or able to survive in the end product. Type 1 rules can for example remove vegetative bacteria for a pasteurised product. Still, type 1 rules do not provide an exclusive list of relevant pathogens. A pasteurised product may be subject to recontamination after inactivation, leading to presence of vegetative pathogens in the end product, and failures in the pasteurisation process may allow survival of vegetative pathogens. Rules of type 1 do not take into account these aspects which do occur in practice. Nevertheless, rules of type 1 provide a list of relevant pathogens under normal and hygienic circumstances.

Type 2 rules select pathogens that are likely to cause problems in the food product in practice. For example, a pathogen that is very rarely transmitted by food is not likely to cause health problems as a result of consuming a food product, and is therefore removed from the list.

Type 3 rules select pathogens that are able to grow or produce toxin in the product. Ability to grow is based on the use of the minimum and maximum growth temperature, pH, and water activity. Other growth determining factors such as nitrite-content, bactericides etc. are not taken into account, which mostly results in worst-case estimations. Selection on growth possibilities is useful for the reason that exposure to pathogens in general is higher if pathogens did multiply in the consumed product than if they did not, which generally results in higher probabilities of food infection and food poisoning. This is confirmed by several dose-response relations of pathogens (31). Not all pathogens have known growth characteristics however, which presents problems for selection on the basis of growth opportunities. It is a fact that the most important pathogens, such as *Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Salmonella spp., Staphylococcus aureus,* and others, do have known growth characteristics. Also, unknown growth characteristics of pathogens may be replaced by known growth characteristics of related pathogens. For example, the

Table 2. Knowledge rules applied in the hazard identification procedure

Type 1: Rules concerning survival of pathogens:

- If pasteurisation occurs in the production process: remove all vegetative bacteria and viruses that contaminated the product before the inactivation
- If sterilisation or radappertisation occurs in the production process: remove all pathogens that contaminated the product before the inactivation
- If drying occurs: remove *Campylobacter* spp. and *Vibrio* spp. that contaminated the product before drying.
- If the brine concentration exceeds 5% (w/w): Remove *Pseudomonas* spp (20).
- If the brine concentration exceeds 10 % (w/w): Remove all pathogens except for *Staphylococcus aureus* and *Listeria monocytogenes* (20,29).

Type 2: Rules concerning general pathogen characteristics:

- Remove exotic pathogens that are not by nature present in your region. For the Netherlands these are: *Coxiella burnetii, Francisella tularensis, Vibrio parahaemolyticus, Vibrio vulnificus* (14).
- Remove pathogens of which exposure is negligible in your region because of effective risk management. For the Netherlands these are: *Brucella* spp., *Mycobacterium bovis, Salmonella typhi, Vibrio cholerae* (14,16)
- Remove micro-organisms of which foodborne pathogenicity is uncertain: *Acetobacter* spp., *Acinetobacter calcoaceticus, Actinomyces* spp., *Aeromonas* spp., *Aeromonas caviae, Aeromonas hydrophila, Aeromonas sobria, Alcaligenes faecalis, Bacillus anthracis, Bacteroides melaninogenicus, Branhamella catarrhalis, Brucella* spp., *Brucella canis, Campylobacter fetus* subsp. fetus, *Chlamydia psittaci, Chlamydia trachomatis, Chromobacterium violaceum, Citrobacter spp., Citrobacter freundii, Clostridium bifermentans, Clostridium cadaveris, Clostridium carnis, Clostridium histolyticum, Clostridium limosum, Clostridium septicum, Clostridium sordellii, Corynebacterium diphteriae, Corynebacterium pseudotuberculosis, Coxiella burnetii, Dermatophilus congolensis, Edwardsiella tarda, Enterobacter spp., Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Erysipelothrix rhusiopathiae, Flavobacterium meningosepticum, Franciscella tularensis, Haemophilus influenzae, Hafnia alvei, Helicobacter pylori, Klebsiella* spp., *Legionella pneumophila, Leptospira* spp., *Morganella morganii, Mycobacterium bovis, Nocardia farcinica, Plesiomonas shigelloides, Proteus* spp., *Proteus mirabilis, Proteus vulgaris, Providencia* spp., *Providencia alcalifaciens, Pseudomonas aeruginosa, Serratia liquefaciens, Serratia marcescens, Stachybotrys atra, Streptobacillus moniliformis, Streptococcus agalactiae, Streptococcus pyogenes, Yersinia pseudotuberculosis* (16,18-20,26,32,33).
- Remove pathogens that rarely cause problems in man: *Brucella canis, Chromobacterium violaceum, Corynebacterium pseudotuberculosis, Coxiella burnetii, Dermatophilus congolensis, Listeria ivanovii, Listeria seeligeri, Listeria welshimeri, Pseudomonas cocovenenans, Streptococcus bovis, Streptococcus dysgalactiae, Streptococcus equisimilis* (16,20,26).

Type 3: Rules concerning growth opportunities of pathogens:

- Remove pathogens that, according to their growth characteristics (based on pH, temperature, and water activity), cannot grow or produce toxin in the end product.

unknown growth characteristics of *Salmonella dublin* can be substituted by the rough growth characteristics of *Salmonella* spp. The non-availability of growth characteristics can therefore be handled, but should be done with caution. By using all types of rules, pathogens are selected that (i) are present and survive in the end product; (ii) are likely to cause health problems in practice; and (iii) are able to grow in the end product. It is important to perform risk assessments for the pathogens selected by these procedures.

If a strict first analysis to determine the most obvious hazards does not result in an answer, a less strict procedure is the next step. The user is free to choose which types of knowledge rules are used in the hazard identification, as there is no rank order of significance for the types of rules.

Some redundancy and inconsistency exists in the knowledge rules. According to the knowledge rule 'Remove microorganisms of which foodborne pathogenicity is uncertain', all species of a genus (for example *Klebsiella* spp.) have to be removed, as well as explicitly mentioned species (for example *Klebsiella pneumoniae*). In this example *Klebsiella pneumoniae* should actually not be mentioned in the knowledge rule. This problem of redundancy is explained in the description of the food database.

The knowledge rules are clearly defined in the hazard identification procedure, and as the definition is explicit, the rules may be criticised, and changed if necessary. Inconsistencies and new developments can therefore be handled easily.

To apply the knowledge rules properly, the hazard identification procedure must be used by experienced microbiologists. Only this will assure an efficient assessment of the most relevant hazards for a product, at each level of detail. The problem of hazard identification is too important and too complex to entrust to a stand alone system. The experienced microbiologist is supported in his decisions by the best use of literature and expert knowledge. Also, the use of literature and expert knowledge may provide the experienced microbiologist with new ideas or renewed insights into products and production processes.

5. Decision supporting identification system for microbial hazards

For practical use it is very convenient to implement the interactive procedure as a decision support system. The literature and expert knowledge used in the hazard identification are captured in three databases: a food database, a pathogen database, and a knowledge database. In the following sections, the databases are described, and subsequently the working of the computer program is explained.

5.1 Food database

The food database introduced by Zwietering et al. (34) contains physical characteristics of products and ingredients, which were derived from the literature. Next to physical characteristics, the food database (Database 1) is extended with information on presence of (groups of) microorganisms, and information on foodborne outbreaks in the past (Table 3), also derived from literature. All foods have an identification code (ID) that determines the position of the food in the product classification tree (Fig. 3). The number of foods is more or less infinite and, as may be expected, not for every product/ingredient information on all the subjects is known. The product classification tree can be used to find a substitute for the missing information. In the tree, products are sorted with respect to their physical properties, so that foods that are grouped together are closely related and information on comparable foods can be used.

Some database records contain redundant information. They contain microorganism genera, including all species, as well as explicitly mentioned species of the genus. For example, for the product raw cow's milk (S.A.A.A.A), the food database contains *Bacillus* spp. (15) as well as *Bacillus cereus* (7,27), and *Bacillus subtilis* (27). Actually, the species should not be mentioned, since they belong to *Bacillus* spp. Species are however explicitly mentioned next to genera in the database as the data come from various references. It is not likely that ICMSF (15), which reported *Bacillus* spp. to be present in raw cow's milk, has studied occurrence of all *Bacillus* spp. in raw cow's milk. Most probably, several species of *Bacillus* have been shown to be present in raw cow's milk, which was briefly indicated by '*Bacillus* spp.'. A study that reports the presence of specific species in a product in general gives more certainty of the actual presence of the species than a report of the presence of a genus.

5.2 Pathogen database

For prediction of microbial spoilage, Zwietering et al. (34) developed an organism database. This organism database has been modified into a database that only contains data on pathogens, as the hazard identification procedure only concerns pathogenic microorganisms (Database 2). Next to names of pathogens, with type and family specification, and pathogen characteristics, there is information on practical relevance of pathogens. An example of the information is shown in Table 4. Non-foodborne pathogens and pathogens that have not been conclusively proven to be foodborne are included since these may cause problems related to food safety in the future.

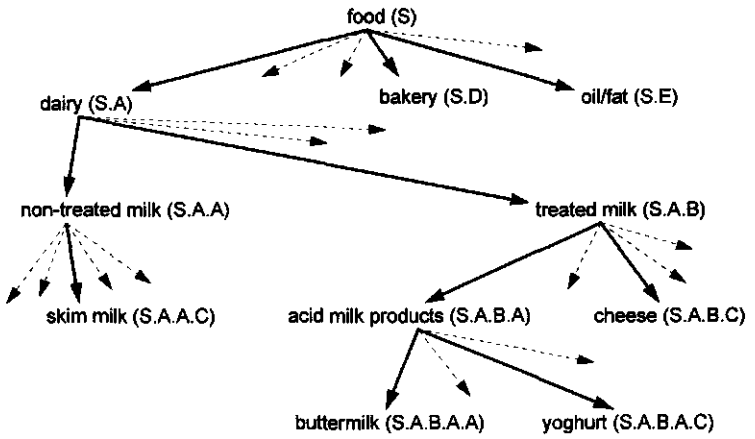

Fig. 3. Structure of food database, in which foods (with identification code) are classified (34).

5.3 Knowledge database

The knowledge database (Database 3) contains knowledge rules. Knowledge rules were developed from the literature, then experts in the field of food microbiology were asked for their opinion on these rules and the rules were changed and reworded accordingly. The knowledge rules stored in Database 3 are shown in Table 2.

5.4 The computer program for hazard identification

The computer program starts with selection of a product and product characteristics, and with construction of a process spreadsheet. After this, the user must choose a level of detail for which the hazard identification procedure will be performed. A list of pathogens is the result of this first selection procedure. The list can be modified according to the user's demands. There are several options of changing the list: add pathogens, remove pathogens, and apply knowledge rules. Addition and removal of pathogens are purely based on the user's expertise. Knowledge rules can be used if the user needs support in shortening the list. The user decides which types of knowledge rules he uses. The knowledge rules belonging to the chosen types appear one by one if appropriate. By acceptance of a knowledge rule, pathogens are deleted from

Table 3. An example of the information stored in the food-database

Name	raw cow's milk
Code	S.A.A.A.A
pH	6.5
Temperature (T)	7
Water activity (a_w)	0.98
Oxygen availability	aerobic
Include groups of microorganisms	Coliforms
Include microorganism	*Actinomyces* spp., *Aeromonas hydrophila*, ..etc
Outbreak related pathogen	*Campylobacter jejuni, Salmonella* spp., ...etc.

Table 4. An example of the information stored in the pathogen database

Name	*Yersinia enterocolitica*
Code	Yers01
Type	bacterium
Spores	no
Infectious	yes
Toxinogenic	no
pH_{min}	4.6
pH_{opt}	7
pH_{max}	9
T_{min}	0
T_{opt}	32
T_{max}	44
$a_{w,min}$	0.97
$a_{w,max}$	1
Oxygen	fac. anaerobic
Food	yes
Exotic	no
Exposure negligible in the Netherlands	no
Pathogenicity uncertain	no
Rarely caused problems	no
No problems in Western countries	no

the list. Before removal however, the computer program provides warnings for several knowledge rules. Amongst the benefits of these warnings is the opportunity to take typical recontamination routes into account. For example, if the knowledge rule 'If pasteurisation occurs: remove all vegetative bacteria and viruses' (Table 2) appears, it can be accepted or neglected. By acceptation *Salmonella* spp. is among the pathogens that are removed from the list. Before the pathogens are removed however, the computer program warns that *Salmonella* spp. may cause problems if the food is of animal origin, because of recontamination by workers' hands (11). If the warning is accepted, the pathogen is not removed.

The outcomes are derived by matching data from the databases. The process of matching data was described by Zwietering et al. (34). If, for example, selection on

growth characteristics (type 3 knowledge rule) is performed, the physical properties of the product in Database 1 are matched to the growth characteristics of pathogens in Database 2.
The final result of the hazard identification procedure is a list of pathogens, that, according to the user and the information from the databases, are hazardous.

6. Results

The hazard identification procedure was applied to vacuum-packed cooked potatoes, cooked ham, and sterilised milk.

6.1 Cooked potato

The results of the first two levels of detail applied to vacuum-packed cooked potatoes are shown in Table 5. First a rough hazard identification was performed, by which pathogens were assessed that were reported to have caused health problems related to cooked potato in the past. The pathogen database found *Clostridium botulinum* type A to be reported to have caused problems in the past in vacuum-packed cooked potatoes. It is prudent to first evaluate the risk of this pathogen in the process, since this organism is likely to be the most obvious hazard. If the risk is assessed for this hazard, and it is found to be acceptable, a more detailed hazard identification should be performed based on pathogens present in the ingredients of cooked potatoes. The ingredients used for the production of vacuum-packed cooked potatoes are potatoes. Also, water is considered to be an ingredient, since potatoes are washed with water during the production process. 32 pathogens were selected to be present in the ingredients potatoes and water (Table 5). Since this list is quite large it is useful to make a selection within this list and first start with the most likely pathogens to cause problems. For this selection knowledge rules can be used. Table 5 shows the results of application of the various types of knowledge rules. Application of type 1 rules resulted in a list of 9 pathogens, application of type 2 rules resulted in a list of 24 pathogens, and application of type 3 rules resulted in a list of 12 pathogens. For application of type 3 rules it was assumed that the pH of cooked potatoes is 6.2 ± 0.1, the water activity is 0.98 ± 0.01 (15), and the temperature is 6 ± 1 ^{0}C, assuming that the potatoes are stored chilled. The ranges in pH, temperature (T), and water activity (a_w) are used to compensate for uncertainties in pH, T, and a_w, of the product and inaccuracies in determining the minimal pH, T, and a_w at which growth can occur.

Table 5. Results of the identification procedure applied to vacuum-packed cooked potatoes and results after application of the three types of knowledge rules

rough hazard identification	***detailed hazard identification***	***knowledge rules***			
		Type 1	***Type 2***	***Type 3***	***Type 1&2&3***
Clostridium botulinum type A	*Aeromonas* spp.			X	
	Alcaligenes spp.		X		
	Bacillus spp.	X	X		
	Bacillus anthracis	X		X	
	Bacillus cereus	X	X	X	X
	Chromobacterium spp.		X		
	Clostridium spp.	X	X		
	Clostridium botulinum type A	X	X		
	Clostridium botulinum type B	X	X		
	Clostridium botulinum type E	X	X	X	X
	Clostridium botulinum type F	X	X	X	X
	Clostridium perfringens	X	X		
	Corynebacterium spp.		X		
	Enterococcus spp.		X		
	Escherichia coli		X	X	
	Flavobacterium spp.		X		
	Klebsiella spp.				
	Klebsiella pneumoniae				
	Listeria monocytogenes		X	X	
	Norcardia spp.		X		
	Pasteurella multocida		X		
	Plesiomonas shigelloides				
	Pseudomonas spp.		X		
	Pseudomonas aeruginosa			X	
	Pseudomonas pseudomallei				
	Salmonella spp.		X	X	
	Serratia spp.		X		
	Shigella spp.		X	X	
	Staphylococcus spp.		X	X	
	Streptococcus spp.		X		
	Vibrio cholerae				
	Yersinia enterocolitica		X	X	

The pathogens left after application of all knowledge rules are *Bacillus cereus*, *Clostridium botulinum* type E, and *Clostridium botulinum* type F. The three pathogens left can be present, and are able to survive and grow in the product. In practice, they may well cause health problems as a result of consuming cooked potatoes. Therefore, it is important to perform risk assessments for these three pathogens according to literature and expert knowledge.

The results show that the databases used are not complete. *Clostridium botulinum* type B was removed from the list because of its growth characteristics. According to the pathogen database the minimal growth temperature (T_{min}) of *Clostridium botulinum* type B is 12.5 ^{0}C. However, T_{min} of *Clostridium botulinum* type B, non-proteolytic strains is 5 ^{0}C (20), which is not in the database. The pathogen database does not take differences in proteolytic and non-proteolytic strains into account, yet. The databases therefore have to be extended and updated regularly.
Due to the clear procedure these types of shortcomings are easily detected and corrected.
It is remarkable that *Clostridium botulinum* type A, which was identified as the most relevant pathogen, was not identified in the detailed hazard identification, when using all types of knowledge rules. *Clostridium botulinum* type A was identified in the detailed hazard identification as present on the ingredients, but it was removed from the list by type 3 knowledge rules. The fact is that *Clostridium botulinum* type A is not able to grow in vacuum-packed cooked potatoes under normal conditions, in this case at a temperature of 6 ^{0}C. Its minimum growth temperature was reported to be 10 ^{0}C (20). The reported outbreak of botulism was most probably caused by storage at temperatures higher than 10 ^{0}C (10). This shows that the detailed hazard identification, including the use of all knowledge rules, only identifies hazards that are relevant under normal, hygienic conditions.

6.2 Cooked ham

The results of the first two levels of detail applied to cooked ham are shown in Table 6. First a rough hazard identification was performed. For the product cooked ham, the pathogen database only found *Clostridium perfringens* that was reported to have caused problems in the past. After a risk assessment for this pathogen is performed and risk is estimated to be acceptable, the hazard identification procedure can be continued with a detailed hazard identification based on the potential presence of pathogens in ingredients. The ingredients used in the preparation of cooked ham are ham and brine. Brine consists of salt, water, and several additives, like spices, ascorbate, and glutamate (5). According to Table 6, 52 pathogens were identified to be present in the ingredients. If knowledge rules were applied type 1 rules resulted in a list of 10 pathogens, type 2 rules in a list of 36 pathogens, and type 3 rules in a list of 12 pathogens (Table 6). To use type 3 rules, it was assumed that the pH of cooked ham is 6.4 ± 0.1, the temperature is 5 ± 1 ^{0}C, and the water activity is 0.98 ± 0.01, based on data from ICMSF (15).

Table 6. Results of the identification procedure applied to cooked ham and results after application of the three types of knowledge rules

rough hazard identification	***detailed hazard identification***	***knowledge rules***			
		Type 1	***Type 2***	***Type 3***	***Type 1&2&3***
Clostridium perfringens	*Acinetobacter* spp.		X		
	Aeromonas spp.			X	
	Aeromonas hydrophila				
	Alcaligenes spp.		X		
	Alcaligenes faecalis				
	Bacillus spp.	X	X		
	Bacillus anthracis	X		X	
	Bacillus cereus	X	X	X	X
	Bacillus subtilis	X	X	X	X
	Brucella melitensis		X		
	Brucella suis		X		
	Campylobacter spp.		X		
	Campylobacter coli		X		
	Campylobacter jejuni		X		
	Chlamydia psittaci				
	Citrobacter spp.				
	Citrobacter freundii				
	Clostridium spp.	X	X		
	Clostridium botulinum type A	X	X		
	Clostridium botulinum type B	X	X		
	Clostridium botulinum type E	X	X	X	X
	Clostridium botulinum type F	X	X	X	X
	Clostridium perfringens	X	X		
	Corynebacterium spp.		X		
	Enterobacter spp.				
	Enterobacter cloacae				
	Enterobacter hafniae				
	Enterococcus spp.		X		
	Erysipelothrix spp.		X		
	Erysipelothrix rhusiopathiae				
	Escherichia spp.		X		
	Escherichia coli		X	X	
	Flavobacterium spp.		X		
	Leptospira spp.				
	Listeria spp.		X		
	Listeria monocytogenes		X	X	
	Moraxella spp.		X		
	Nocardia spp.		X		
	Plesiomonas shigelloides				
	Proteus spp.				
	Pseudomonas spp.		X		
	Pseudomonas aeruginosa			X	
	Salmonella spp.		X	X	
	Salmonella anatum		X		
	Salmonella montevideo		X		
	Serratia spp.		X		
	Serratia liquefaciens				

Table 6 continued:

Staphylococcus spp.	X	
Staphylococcus aureus	X	X
Streptococcus spp.	X	
Yersinia spp.	X	
Yersinia enterocolitica	X	X

If all types of knowledge rules are applied to shorten the list, only four pathogens are left: *Bacillus cereus, Bacillus subtilis*, and *Clostridium botulinum* type E, and type F. It is sensible to firstly perform risk assessments for these pathogens. However, as mentioned before, selection on growth possibilities is based only on minimum and maximum temperature, pH, and water activity. Inhibitory effects of the nitrite in the brine, which are very important for the safety of cooked ham, are not taken into account. Also, the expert knowledge in the computer program is general expert knowledge, and therefore no specific expert knowledge on bacteria in cooked ham is available. The user needs to have specific knowledge, and based on his experience in the specific situation, the user may not apply all knowledge rules. He may have strong arguments to delete *Bacillus subtilis* or *Bacillus cereus* from the list, or add other pathogens to the list.

Still, the hazard identification procedure identifies hazards that are the most likely to cause problems under normal, hygienic conditions. Therefore the hazard identification procedure may be considered to provide a good start for performing risk assessments for cooked ham.

6.3 Sterilised cow's milk

The last product for which a hazard identification was conducted is sterilised cow's milk. The rough hazard analysis did not result in identification of a pathogen that was reported to have caused health problems related to sterilised cow's milk in the past. Continuing with the detailed hazard analysis, 62 pathogens were identified as present on the ingredient raw cow's milk. Application of type 1 rules resulted in identification of zero hazards. This is related to the confirmation of the knowledge rule concerning sterilisation (Table 2), which removed all pathogens. Application of type 2 knowledge rules resulted in a list of 43 pathogens, and application of type 3 rules identified 14 pathogens as hazardous. It was assumed that the pH of milk is 6.5 $\pm$ 0.1, that the water activity is 0.98 $\pm$ 0.01 (15), and that the temperature is 6 $\pm$ 1 ^{0}C

Table 7. Results of the identification procedure applied to sterilized cow's milk and results after application of the three types of knowledge rules

rough hazard identification	*detailed hazard identification*	*knowledge rules*			
		Type 1	*Type 2*	*Type 3*	*Type 2&3*
***	*Acinetobacter* spp.		X		
	Actinomyces spp.				
	Aeromonas spp.			X	
	Aeromonas hydrophila				
	Alcaligenes spp.		X		
	Bacillus spp.		X		
	Bacillus cereus		X	X	X
	Bacillus subtilis		X	X	X
	Brucella spp.			X	
	Brucella abortus		X	X	X
	Brucella melitensis		X	X	X
	Brucella suis		X		
	Campylobacter spp.		X		
	Campylobacter coli		X		
	Campylobacter jejuni		X		
	Chromobacterium spp.		X		
	Citrobacter spp.				
	Clostridium spp.		X		
	Clostridium butyricum		X		
	Clostridium perfringens		X		
	Corynebacterium spp.		X		
	Corynebacterium bovis		X		
	Corynebacterium pyogenes		X		
	Coxiella burnetii				
	Cryptococcus neoformans		X		
	Enterobacter spp.				
	Enterobacter aerogenes				
	Enterobacter cloacae				
	Enterococcus spp.		X		
	Enterococcus faecalis				
	Escherichia spp.		X		
	Escherichia coli		X	X	X
	Flavobacterium spp.		X		
	Leptospira spp.				
	Listeria spp.		X		
	Listeria monocytogenes		X	X	X
	Moraxella spp.		X		
	Mycobacterium spp.		X		
	Mycobacterium bovis				
	Mycobacterium tuberculosis		X		
	Mycoplasma spp.		X		
	Nocardia spp.		X		
	Nocardia asteroides		X		
	Pasteurella multocida		X		
	Proteus spp.				
	Pseudomonas spp.		X		
	Pseudomonas aeruginosa			X	
	Salmonella spp.		X	X	X

Table 7 continued:

Salmonella dublin	X		
Salmonella typhi		X	
Salmonella typhimurium	X		
Staphylococcus spp.	X	X	X
Staphylococcus aureus	X	X	X
Staphylococcus epidermidis	X		
Streptobacillus moniliformis			
Streptococcus spp.	X		
Streptococcus agalactiae			
Streptococcus bovis			
Streptococcus dysgalactiae			
Streptococcus equisimilis			
Streptococcus pyogenes			
Streptococcus zooepidemicus	X		
Yersinia spp.	X		
Yersinia enterocolitica	X	X	X

*** No organisms were found in the database that were reported to have caused health problems related to sterilised cow's milk

(sterilised milk is normally cooled after opening of the carton). Combination of the three types of knowledge rules resulted in zero hazards of course, because of the negative result of the application of type 1 rules. Combination of type 2 and type 3 rules however resulted in a list of 10 pathogens. These pathogens are relevant in case the sterilising process is not properly controlled and in case recontamination of milk occurs after sterilisation. The user's knowledge is important to apply this list, which resulted mainly from literature and expert knowledge, for his specific situation.

7. Conclusion

A hazard identification procedure was developed and implemented as a computer program, to perform systematically the first step of quantitative risk analysis. The hazard identification procedure was based on the general approach for hazard identification presented by Notermans et al. (24). It differs from Notermans' approach by its stepwise identification of important hazards and its interactive character.

Relevant hazards are identified stepwise by the use of several levels of detail. The levels are: rough hazard identification, detailed hazard identification, and comprehensive hazard identification. First, the level of least detail is used to identify the most obvious hazards. For these hazards, risk assessment studies should be performed first. If the calculated risk is acceptable, risk assessments can be carried out

for less relevant hazards. Risk assessments should not stop when the most important problems are controlled. As mentioned, risk assessments for less relevant hazards should be performed consecutively.

The interactive character results from the use of several knowledge sources in hazard identification. The knowledge sources are: literature knowledge, expert knowledge and the user's knowledge. By the use of literature knowledge only, theoretical hazards are identified that may not be relevant in certain cases. These theoretical hazards can be treated with relativism by the use of expert knowledge, captured in knowledge rules. Three types of knowledge rules were developed, that can be used in combination or apart from each other. The knowledge rules are clearly defined in the hazard identification procedure, and as the definitions are explicit, the knowledge rules may be criticised, and changed if necessary. By the use of knowledge rules, a well founded way is provided to remove theoretical hazards, that are not relevant in specific cases. However, expert knowledge is mostly general knowledge, and therefore the user's knowledge is used to focus on those hazards that are most relevant in specific situations. The interactive character of the procedure implies that the procedure does not give definite answers on microbial hazards in food products. The hazard identification procedure is therefore best used by experienced microbiologists, who are supported in their decisions by the best use of literature and expert knowledge. Thus, the most relevant hazards in a product may be assessed efficiently, at three levels of detail.

Implementation of the hazard identification procedure as a computer program resulted in a decision supporting identification system which uses several databases to identify relevant hazards for certain products. The databases are not complete. This is inevitable, for it is not possible to describe all possible products and ingredients, nor is it possible to describe all existing pathogens, with all related foodborne outbreaks and all related ingredients etc. However, the databases do contain much information to perform reliable hazard identifications. In order to improve hazard identifications in future, the databases should be updated regularly. It is also possible to combine databases, related to quantitative risk analysis, from all over the world. By this combination, much unnecessary work to extend databases can be prevented. This approach may finally result in a generally applicable hazard identification system and a structured method of collection of literature data.

In future, the hazard identification procedure and decision support system will be part of a general procedure for quantitative risk assessments for food products. As well as the hazard identification procedure, the procedure for quantitative risk as-

sessment should be based on the use of three levels of detail and the combination of different knowledge sources.

The hazard identification procedure described above is the first step of a procedure for quantitative risk assessments that has been developed as a computer-aided system. This has resulted in a complete decision support system for quantitative risk assessment of microbial contamination of food products.

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3

Growth and inactivation models to be used in quantitative risk assessments

Abstract

In past years, many models describing growth and inactivation of microorganisms have been developed. This study is a discussion of the growth and inactivation models that can be used in a stepwise procedure for quantitative risk assessment. First, rough risk assessments are performed in which orders of magnitude for microbial processes are estimated by the use of simple models. This provides an efficient way to find the main determinants of risk. Second, the main determinants of risk are studied more accurately and quantitatively. It is best to compare several models at this level, as no model is expected to be able accurately to predict microbial responses under all circumstances. By comparing various models the main determinants of risk are studied from several points of view, and risks can be assessed on a broad basis. If, however, process variations have a more profound effect on risk than the differences between models, it is most efficient to use the simplest model available. If relevant, the process variations can be stochastically described in the third level of detail. Stochastic description of the process parameters will however not change the conclusion on the usefulness of simple models in quantitative risk assessments. The proposed stepwise procedure that starts simply before going into detail provides a structured method of risk assessment and prevents the researcher from getting caught in too much complexity. This simplicity is necessary because of the complex nature of food safety. The principal aspects are highlighted during the procedure and many factors can be omitted since their quantitative effect is negligible.

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1. Introduction

Food-borne infection and food poisoning are serious problems for human health. In the past years many food production companies have been working on safety management systems systematically to prevent outbreaks of food infection and poisoning. A quantitative approach to food safety control can be created by development and implementation of quantitative risk assessment for food products in existing safety management systems. Quantitative risk assessment for food products has been described by several authors (18,23,29,41).

A procedure for stepwise quantitative risk assessment has been developed. The stepwise approach uses three levels of detail, varying from qualitative, rough risk assessments to detailed quantitative risk assessments. This approach allows identification of the most relevant problems before focusing on less important problems. Following this procedure is necessary to efficiently assess risks in the complex context of food safety.

The purpose of this study is to discuss growth and inactivation models to be used in a stepwise procedure for quantitative risk assessment.

1.1 Stepwise procedure for quantitative risk assessment

The procedure to systematically perform quantitative risk assessments is based on the use of three levels of detail: level 1, semi-quantitative risk assessment; level 2, quantitative deterministic risk assessment; and level 3, quantitative detailed risk assessment.

First the procedure must be conducted roughly and mainly qualitatively (level 1) to initially identify the scope of the most important hazards, the risk-determining process steps, and risks. The results of level 1 are used in level 2. In level 2 both specific models and/or general models can be used to quantitatively describe the risk-determining phenomena. The results of the models can then be compared, to estimate risk on a broad basis. Also in level 2, effects of possible changes in process or product parameters (for example by failures) can be estimated. The results of level 2 can be used in level 3, which is the most detailed level, to perform calculations and simulations using, for instance, very detailed and specific models or stochastic variables. The latter are useful if process variations determine risk to a great extent.

Figure 1 shows a schematic representation of a procedure for microbiological quantitative risk assessment. In the first step (hazard, product, and process identification), the microbiological hazards related to the product are assessed, the product and the production process are described, and product and process characteristics are gathered.

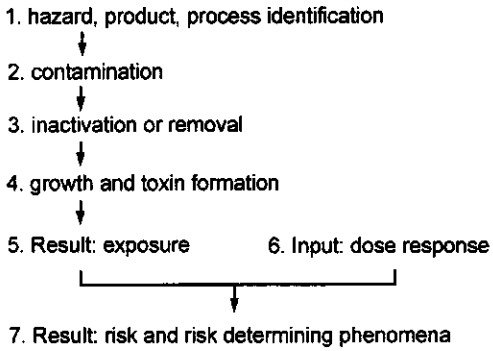

Fig. 1. Schematic representation of a procedure for quantitative risk assessment.

In the second step, the probability of contamination, the magnitude of contamination, and the spatial distribution of contamination are described.

If there is an inactivation step (like heat treatment or irradiation) or removal step (like peeling or washing) that inactivates or removes the contamination, in the third step the extent of inactivation or removal is estimated. Reduction by a factor of 10^{20} or higher was assumed to be complete inactivation. Inactivation up to a factor 10^{1} was assumed not to be a relevant inactivation.

In the fourth step, growth and toxin formation are estimated. If incomplete inactivation occurs in the production process, both growth and toxin formation before and after the inactivation are relevant. If complete inactivation occurs, only toxin formation before inactivation is relevant if heat-stable toxins are concerned.

The fifth step of the procedure, the exposure assessment, combines the results of the foregoing steps.

The sixth step of the procedure is the dose-response assessment. Dose-response data are necessary to estimate the probability of infection and illness as a result of consuming a certain amount of pathogenic organisms.

Finally, in the seventh step of the procedure, the dose-response data of step 6 are coupled to the exposure data of step 5, to characterise the risk of illness as a result of consuming the specified product. The main risk-determining phenomena are revealed by following the steps of the procedure.

Table 1. Models to describe the growth curve of microbial cells under stationary conditions

Growth curve	*Equation*
Exponential	$\ln(N) = \ln(N_0) + \mu t$
Lag-exponential	$\ln(N) = \ln(N_0)$, for $t < \lambda$ $\ln(N) = \ln(N_0) + \mu(t - \lambda)$, for $t \geq \lambda$
Logistic (48) *	$\ln(N) = \ln(N_0) + \frac{a}{[1 + \exp(b - cx)]}$
reparameterised Gompertz (48) †	$\ln(N) = \ln(N_0) + A\exp\left\{-\exp\left[\frac{\mu_{max}e}{A}(\lambda - t) + 1\right]\right\}$
Baranyi (4) †‡	$\ln(N) = \ln(N_0) + \mu_{max}A_n(t) - \ln\left(1 + \frac{\exp(\mu_{max}A_n(t)) - 1}{\exp(A)}\right)$
Jones (20) *§	$\ln(N) =$ $\ln(N_0) - \ln(2)\left(\exp\left[\frac{t-d}{c}\right] - \exp\left[\frac{-(t-d)}{c}\right] - \exp\left[\frac{-d}{c}\right] + \exp\left[\frac{d}{c}\right]\right) +$ $\ln(2)a\left(1 - \left(1 + \frac{t}{b} + \frac{1}{2}\cdot\left(\frac{t}{b}\right)^2 + \frac{1}{6}\cdot\left(\frac{t}{b}\right)^3\right)\exp\left(\frac{-t}{b}\right)\right)$
Probability (42) ‖	$P(t) = \frac{P_{max}}{(1 + \exp[k(\tau - t)])}$

* a, b, and c are fit parameters.

† μ_{max} is the maximum specific growth rate (h^{-1}), A is the maximum level of increase: $\ln(N_\infty/N_0)$.

‡ A_n as defined by Baranyi *et al.* (4).

§ d is a fit parameter.

‖ $P(t)$, probability of growth at time t; P_{max}, maximum probability; k, rate constant; τ, time to the midpoint of the function.

1.2 Predictive models

In predictive modelling there are primary, secondary, and tertiary models (5). Primary models describe the growth or inactivation curve, or probability of growth; secondary models describe the kinetic parameters of primary models in terms of environmental conditions; tertiary models integrate data for all aspects of responses of microbes to their environment into expert systems or decision support systems. In this way the microbial safety of foods can be efficiently assessed. In this chapter the focus will be on primary and secondary models. The following sections describe which growth and thermal inactivation models can best be used in the several levels of detail of stepwise risk assessment. First, primary and secondary growth models and second primary and secondary inactivation models are discussed. Assuming that most models fit growth data well and are statistically acceptable, practical considerations were taken into account. Some practical considerations are the simplicity of the models, the ability to look up parameters in the literature and databases, practical applicability, biological meaning of the parameters, limits of growth, and the number of parameters.

2. Primary growth models

The simplest way to describe growth is by assuming first-order kinetics. Growth can then be described by an exponential function. To include the lag time (λ), growth can be described by the lag-exponential function (Table 1). Bacterial growth is also often described by sigmoidal curves. Several sigmoidal functions used to describe the growth curve empirically are the logistic, Gompertz, Richards, Schnute, and Stannard (48). In later studies less empirical models were developed, based on bacterial life cycles (4,16,20,43). Some of the functions and models are shown in Table 1.

For pathogens with zero-growth tolerance like *Clostridium botulinum, Salmonella*, and *Listeria* spp., models for description of the entire growth curve are not appropriate according to Baker and Genigeorgis (2) and Whiting and Call (42). Probability models were developed to describe the probability of one spore or vegetative cell initiating growth and toxigenesis (Table 1).

Most of the models shown in Table 1 are empirically used equations or analytical solutions of differential equations, describing the number of microorganisms in time under constant environmental conditions. To describe the amount of microorganisms under changing conditions, differential equations are needed; the growth curve can be generated using numeric calculations. This was shown for the Baranyi and Gompertz models respectively by, for instance, Baranyi and Roberts (4) and Van

Impe *et al.* (39). The differential equation for the lag-exponential model can be deduced by assuming first-order kinetics for a batch system: $dN/dt = \mu N$, for $t > \lambda$.

2.1 Primary growth models to be used in the first rough level of detail

For rough risk assessments, orders of magnitude for growth can easily be estimated by using the exponential growth function, neglecting lag time (λ) and stationary growth (Table 1). The assumption of $\lambda = 0$ results in fail-safe predictions. Stationary growth is generally not relevant in risk assessments, as the product may indeed be spoiled at that stage, and for pathogens this level is definitely too high.

On the basis of the estimated order of magnitude it can be decided whether growth is one of the main determinants of risk.

2.2 Primary growth models to be used in the second quantitative level of detail

If growth is one of the main determinants of risk, it can be useful to describe the entire growth curve in level 2. As stationary growth is generally not much of interest in risk assessments, growth can be simply described by the lag-exponential function (Table 1). In quantitative risk assessments it is best, however, not to rely on the results of one model only. If possible and relevant, several models should be used and their results compared to make reliable decisions on risks.

For general predictive purposes, the Gompertz (14,48) and Baranyi (4) models have an important practical advantage over most other sigmoidal models (16,20,43) and probability models (25,42); the biologically interpretable parameters of the Gompertz (14,48) and Baranyi (4) models have been described in relation to environmental factors (in secondary models) by many studies. The Baranyi model is less empirical than the Gompertz function, and an important disadvantage of the Gompertz function is that it does not give exactly $N = N_0$ at $t = 0$. For relatively short processes the lack of this information may have significant effects on predicted growth.

Considering the above reasons, it was decided that the lag-exponential function and the Baranyi model in level 2 were preferable, if the results of level 1 showed growth to be a main determinant of risk. If specific models for certain situations are available these can be used as well.

If however process variations are of much more importance than differences in model predictions, comparison of several model predictions will not substantially contribute to a broad view on risk. In that case it is preferable to use the simplest model available (here the lag-exponential model), an indication of the usefulness of simple models in advanced risk assessments.

3. Secondary growth models

Secondary kinetic models can be divided into four main model types: (i) square root (27,30,34,44,46); (ii) Arrhenius/Eyring (1,35); (iii) linear Arrhenius-Davey (11); and (iv) polynomial or response surface models (6,26,38). The model types have been comprehensively described in a review paper on predictive microbiology by Ross and McMeekin (33). Examples of the secondary model types are shown in Table 2.
Many validation studies have been performed comparing the various types of secondary models. Several studies have shown varying results (40). This is confirmed by the fact that it is often difficult to compare models, since the models do not always contain the same controlling factors (13).

3.1 Secondary models to be used in the first, rough level of detail

In level 1, specific growth rate is described by the gamma model (Table 2) (46). The gamma model can generally be applied, since the parameters can be found in the literature for many pathogens. Moreover, the gamma model is simple in structure, easy to interpret, and has few parameters. The gamma model is a square root type of model, and uses dimensionless growth factors to calculate the relative effects of environmental variables on the specific growth rate. The growth factors are defined for pH, water activity (a_w), and temperature (T). New variables can easily be included in the model. The gamma model provides an efficient way to obtain a quantitative impression of the specific growth rate and quantitative insight into the relevance of several environmental conditions for growth.

3.2 Secondary models to be used in the second, quantitative level of detail

Apart from the specific growth rate, both the lag time (λ) and the maximum amount of pathogens (N_∞) have to be estimated in level 2. Zwietering *et al.* (45) showed that λ is often reciprocally proportional to μ, and a general value of $N_\infty = 10^9$ CFU was given by Buchanan *et al.* (6, 7).
Considering the practical advantages of parameter availability and the biological meaning of the parameters (Table 3), it was decided to use first square root models in level 2. For λ, square root models that use temperature effects only are used: these result in worst-case estimates.

Table 2. Secondary model types for growth rate in predictive microbiology

Model type	*Equation*
Square root	$\sqrt{\mu} = b(T - T_{\min})\sqrt{(a_w - a_{w\min})}\sqrt{(pH - pH_{\min})}$
Square root: Gamma model (46)	$\mu = \mu_{opt} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w)$ with $\gamma(T) = \left(\frac{T - T_{\min}}{T_{opt} - T_{\min}}\right)^2$ $\gamma(pH) = \frac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})}$ $\gamma(a_w) = \frac{a_w - a_{w\min}}{1 - a_{w\min}}$
Arrhenius/Eyring (35) *	$\mu = \frac{\rho_{25}\frac{T}{298}\exp\left\{\frac{H_A}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right\}}{1 + \exp\left\{\frac{H_L}{R}\left(\frac{1}{T_{1/2L}} - \frac{1}{T}\right)\right\} + \exp\left\{\frac{H_H}{R}\left(\frac{1}{T_{1/2H}} - \frac{1}{T}\right)\right\}}$
Linear Arrhenius-Davey (11) †	$\ln(\mu) = a + \frac{b}{T} + \frac{c}{T^2} + da_w + ea_w^2$
Polynomial	$\log(\mu) = a + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n}\sum_{j=i}^{n} b_{ij} x_i x_j$

* R is the universal gas constant and ρ_{25}, H_A, H_L, H_H, $T_{1/2L}$, and $T_{1/2H}$ are identified by Schoolfield *et al.* (35). T in degrees Kelvin.

† a, b, c, d, and e are fit parameters. T in degrees Kelvin.

Table 3. Advantages and disadvantages of various secondary models

Advantages	***Disadvantages***
Square root	
• biological interpretable parameters • parameters can be found in literature • for every variable relative effect can be calculated • easy to interpret • based on curvature of various variables	• non-linear regression if pH and/or a_w are included • no theoretical foundation • parameters extrapolated to growth limits
Arrhenius/Eyring (Schoolfield)	
• biological meaning parameters	• non-linear regression • parameters often used as fit parameters, instead of estimates of biologically relevant parameters • does not predict limiting values for variables
Linear Arrhenius-Davey	
• linear regression	• no biological meaning parameters • does not predict limiting values for variables
Polynomial	
• linear regression • straightforward • no knowledge of process needed	• no theoretical foundation • no parameters with biological meaning • often no experiments at growth limits • interpolation within ranges not always possible • uses many parameters: may lead to description of errors as well • only applicable to the situation for which it was developed • does not contribute to knowledge about mechanisms • numerous parameters • no extrapolation possible

Table 4. Models used to describe the inactivation curve

Inactivation curve	*Equation*
Exponential	$\ln(N) = \ln(N_0) - kt$ with $k = \frac{2.303}{D}$
Exponential-tailing *	$\ln(N) = \ln(N_0) - kt$, for $t < t_\infty$ $N = N_\infty$, for $t \geq t_\infty$ with $t_\infty = -\frac{\ln\left(\frac{N\infty}{N_0}\right)}{k}$
Vitalistic theory: • logistic (10) †	$\log(N) = \alpha + \frac{\omega - \alpha}{1 + \exp\left[4\sigma \frac{\tau - \log(t)}{\omega - \alpha}\right]}$
Mechanistic theory: • Activated & dormant spores‡ (32). Mainly focused on description of shoulder.	$N = (N_{0a} + N_{0d})\exp\left[\frac{-t}{\tau_i}\right] - N_{0d}\exp\left[\frac{-t}{\tau_{ai}}\right]$
• Different bacterial populations § (22). Mainly focusing on tailing	$\log(N) = \log(N_0) + \log\left(\frac{2F_1}{1+\exp[k_1 t]} + \frac{2(1-F_1)}{1+\exp[k_2 t]}\right)$
• Gompertz, empirical ‖ (24)	$\log(N) = \log(N_0) + a\exp[-\exp(b+ct)] - a\exp[-\exp(b)]$

* N_∞/N_0 is the maximum level of reduction.

† α, upper asymptote; ω, lower asymptote; τ, position of maximum slope; σ , maximum slope.

‡ N_{0a} and N_{0d} are initial population sizes of activated spores and dormant spores respectively; τ_i , time constant for inactivation; τ_{ai}, combined time constant for inactivation and activation.

§ F_1 and $1\text{-}F_1$ represent the two fractions of bacteria, and k_1 and k_2 are the specific inactivation rates for the two fractions.

‖ a, b, and c are fit parameters.

To prevent total dependence on the extrapolated minimum and maximum growth characteristics (as used in the square root models) and to be able to compare several models, specific models can be used, if appropriate. Several polynomial models have been implemented in the USDA (U.S. Department of Agriculture) Pathogen Modeling Program version 5.0, and can therefore easily be used. The use of models takes only a little time, and therefore comparisons are easily made.
It must be kept in mind that lag time is very difficult to model, as it is strongly dependent on the organism (its history and physiological state) and the food product (47). The use of more specific models like Pathogen Modeling Program might therefore be justified, but may not describe reality; the predicted lag time can be totally different from reality.

4. Primary inactivation models

For many years, thermal inactivation has been described by first-order kinetics. Using first-order kinetics, the rate of inactivation (k, min^{-1}) is described by the slope of the inactivation curve on a semi-logarithmic plot. The use of the rate constant k as the inactivation parameter is preferred, because k is a variable that is widely used in several disciplines (chemical reactions, enzymatic reactions, etc.). The use of k makes comparisons with other disciplines possible and is a more general concept than other inactivation parameters. In the field of microbiology, the decimal reduction time (D) is often used as the inactivation parameter. On the basis of its definition and assuming first-order inactivation kinetics, D can easily be recalculated as k (Table 4).
In past years other modelling approaches for thermal inactivation were developed that describe the often-found significant deviations from loglinear inactivation. Two main theories exist in inactivation modelling: the vitalistic and the mechanistic concept. These concepts were extensively reviewed by Cerf (9). Some examples of primary heat-inactivation models are shown in Table 4.

4.1 Primary inactivation models to be used in the first, rough level of detail

In level 1, orders of magnitude for thermal inactivation are estimated assuming first-order inactivation. Nonthermal inactivation (for example irradiation) can also be described by first-order inactivation kinetics in level 1. Nonthermal inactivation will not be discussed specifically in this chapter. Shoulders and tailing phenomena are neglected in level 1, which generally does not affect qualitative conclusions on the relevance of inactivation for risk.

Table 5. Secondary models to describe inactivation rate

Model type	*Equation*
Arrhenius *	$k = k_{\infty} \exp\left(\frac{-E_a}{RT}\right)$
Eyring (31) †	$k = k_O + \kappa \frac{k_b T}{h} \cdot \left(\exp\left[\frac{\Delta G^*_H}{RT}\right] \cdot \left[H^+\right]^{nH} + \exp\left[\frac{\Delta G^*_{OH}}{RT}\right] \cdot \left[OH^-\right]^{nOH}\right)$
Linear Arrhenius-Davey (12) ‡	$\ln(k) = a + \frac{b}{T} + \frac{c}{pH} + \frac{d}{pH^2}$
Polynomial (21)	$\log\left(\frac{2.303}{k}\right) = \log(D) = a + b_1 T + b_2 pH \ldots + b_z T^2$
z-concept §	$\log\left(\frac{D_r}{D}\right) = \frac{T - T_r}{z}$ $z = \frac{2.303 RTT_r}{E_a}$

* k_∞ is the rate constant at infinite temperature, E_a is the activation energy, R is the universal gas constant; T in degrees Kelvin.

† k_o, κ, k_b, h, n_H, n_{OH}, ΔG_H^* and ΔG_{OH}^* as defined by Reichart (31); T in degrees Kelvin.

‡ a, b, c, and d as fit parameters; T in degrees Kelvin.

§ T in degrees Kelvin.

4.2 Primary inactivation models to be used in the second, quantitative level of detail

If inactivation appears to be relevant for risk, it can be useful to describe deviations from first-order inactivation. Especially tailing-off phenomena may produce dramatic increases of apparent D (8).

The main practical problem of the present thermal inactivation models describing tailing phenomena and shoulders is however that they cannot be used for general predictive purposes, because of the lack of parameter values for other than reported cases.

For the above reason we have chosen to describe thermal inactivation by first-order inactivation kinetics in level 2 as well. Tailing can be taken into account by assuming a maximum level of inactivation; for example, a sixfold reduction at maximum. For this operation the exponential-tailing model (Table 4) can be used. Shoulders can be taken into account by assuming a lag time for inactivation; the lag-exponential model (Table 1) can be used. For inactivation, μ in Table 1 is not the growth rate (h^{-1}), but the inactivation rate k, (h^{-1}). If specific models for certain situations are available these can be used as well, providing a way to compare results of several models.

5. Secondary inactivation models

The temperature dependence of the inactivation rate (k) is widely assumed to be described by the Arrhenius equation. Several secondary inactivation models have been developed, relating k or other inactivation parameters to environmental factors. The model types resemble secondary-growth model types. Examples of the model types shown in Table 5 are (i) Arrhenius/Eyring (31); (ii) linear Arrhenius-Davey models (12); (iii) polynomial models (10,21,24); and (iv) the D,z concept (3,19,37).

In microbiology, z is often used to indicate the change of temperature necessary for a 10-fold change in reaction rate. D and z values were frequently reported under various circumstances for many pathogens. Most of the studies however, did not quantitatively relate D and z values to environmental factors. Moreover, the parameters of the other model types are often very specific. The use of secondary models is therefore largely restricted.

5.1 Secondary inactivation models to be used in the first, rough level of detail

Orders of magnitude for k are estimated from reported values for D (Table 4) and z (Table 5). The use of secondary models is presently too restricted, and it is not necessary, since orders of magnitude are available from the literature.

5.2 Secondary inactivation models to be used in the second, quantitative level of detail

As in level 1, k is calculated from reported values for D and z. Specific values are used if available; otherwise worst-case estimates are used. If specific values for the parameters of the other secondary inactivation models are available, the models will be used in level 2. Comparisons between reported and predicted values of k are then possible.

Table 6. Rough quantitative risk assessment applied to vacuum-packed cooked potatoes.

Level 1 risk assessment
step 1: hazard, product, process identification Product: Vacuum-packed cooked potatoes. Product characteristics: pH = 6.6, T = 6, and a_w = 0.99. Process steps and parameters: see Table 7. Hazard: *Bacillus cereus* *
step 2: contamination Assumption: all packages are contaminated; there is no recontamination.
Step 3: inactivation or removal Estimated extent of inactivation (step 2,3, and 7, Table 7): $N/N_0 = 0.88$.
step 4: growth and toxin formation Estimated increase: $N/N_0 = 4.5 \cdot 10^6$ (Table 7)
step 5: exposure assessment Exposure: high, ($\approx N_0 \cdot 10^6$)
step 6: dose-response assessment Generally *B. cereus* poisoning and infections if consumption > 10^6 (ranging from 10^4 to 10^8) CFU/g or ml (15).
Step 7: risk and risk-determining phenomena Risk: high Risk-determining phenomena: storage, cooling after pasteurisation, probability of contamination

* *Bacillus cereus* has been reported to be present on potatoes (28). It survives the pasteurisation process, and is able to grow on the product (based on the product characteristics and pathogen characteristics (17,28,36)).

6. Results

6.1 Level 1 risk assessment: vacuum packed cooked potatoes

The stepwise procedure for quantitative risk assessment (Fig. 1) was applied to vacuum-packed cooked potatoes at the first two levels of detail for *Bacillus cereus*, as an example to show the use of growth and inactivation models in exposure assessments.

The results of the first level of detail are shown in Table 6. The extent of inactivation of *B. cereus* was estimated to be $N/N_0 = 0.88$. The parameter k for *B. cereus* was estimated based on reported D and z values (17). In this case it does not matter what the exact degree of inactivation is, since the qualitative conclusion is that no relevant inactivation occurs (less than a factor of 10) for this organism. The estimated increase of *B. cereus* was $4.5 \cdot 10^6$. The growth factors for T, pH, and a_w show that temperature is a restricting factor for growth (minimum value for $\gamma(T)$ at 5 ^{0}C = 0.0034) (Table 7), whereas pH and a_w scarcely influence growth: $\gamma(pH) = 0.99$ and $\gamma(a_w) = 0.86$ respectively (Table 7). Exposure to *B. cereus* was estimated to be high ($\approx N_0 \cdot 10^6$), because of the growth opportunities. Even if $N_0 = 1$, very high numbers of CFU might be formed. On the basis of the high exposure and the dose-response data, risk was estimated to be high. Much growth of *B. cereus* can occur, which may lead

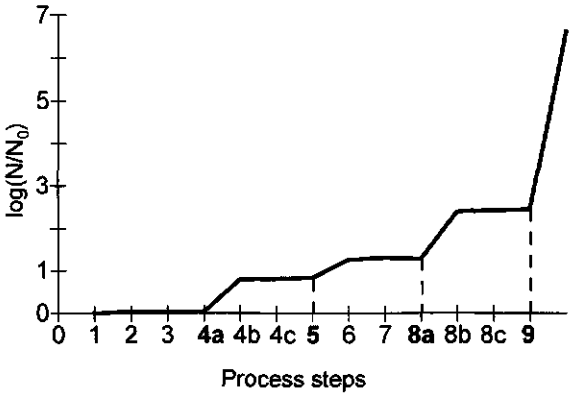

Fig. 2. Estimated increase ($\log(N/N_0)$) in number of *Bacillus cereus* cells in vacuum-packed cooked potatoes during the production process at various process stages, expressed in stage numbers (Table 7). Level 1 estimations are shown.

Table 7. Production process of vacuum-packed cooked potatoes and predicted growth and inactivation of *Bacillus cereus* *

			Growth			*Inactivation*			*Total*
process steps	**temp. (°C)**	**time (h)**	$\gamma(T)$	μ (h^{-1})	N/N_0 †	k (h^{-1})	$10^{-5} \cdot E_a$ (J/molK)	N/N_0 ‡	N_{total}/N_0
1. washing	20	0.25	0.25	0.43	1.1				1.1
2. steam pealing	85	0.025				0.65	2.5	0.98	1.1
3. blanching	65	0.1				0.0068	2.4	1	1.1
4a. cooling	37	1	1	1.71	5.5				6.0
4b. cooling	10	1	0.042	0.072	1.1				6.5
4c. cooling	5	8	0.0034	0.0059	1.0				6.8
5. storage	5	168	0.0034	0.0059	2.7				18
6. packing	20	0.25	0.25	0.43	1.1				20
7. pasteurisation	90	0.05				2.0	2.5	0.90	18
8a. cooling	37	1.5	1	1.71	13				238
8b. cooling	10	1.5	0.042	0.072	1.1				265
8c. cooling	5	9	0.0034	0.0059	1.1				279
9. storage	6	720	0.0078	0.013	$1.4 \cdot 10^4$				$4.0 \cdot 10^6$

* Based on the gamma model and the product parameters: pH = 6.6 and a_w = 0.99. The growth parameters were assumed to be: T_{min} = 3 °C; T_{opt} = 37 °C; T_{max} = 55 °C; pH_{min} = 4.9; pH_{opt} = 6.5; $a_{w,min}$ = 0.93 (17,28,36). The gamma model assumes symmetry for estimation of $\gamma(pH)$, so pH_{max} was calculated to be 8.1. μ_{opt} was assumed to be 2 h^{-1}. As the pH and a_w of the product were assumed to be constant, $\gamma(pH)$ and $\gamma(a_w)$ are constant during the production process: 0.99 and 0.86 respectively.

† $N/N_{o\ total} = 4.5 \cdot 10^6$.

‡ $N/N_{o\ total} = 0.88$.

to consumption of infectious or toxic levels. Most growth occurred during the last stage of the production process: storage (step 9, Table 7 & Fig. 2). Also, much growth occurs during cooling after pasteurisation (step 8a, Table 7 & Fig. 2). Storage and cooling were therefore estimated to be important determinants of risk. Also, the percentage of potatoes contaminated is important; if potatoes are rarely contaminated, risk is much lower. These three aspects should be considered in the second level of quantitative risk assessments. The inactivation step and many other steps were estimated not to be relevant for risk. These steps will therefore not be considered in further analysis.

6.2 Level 2 risk assessment: growth, Bacillus cereus

In level 2, all main determinants of risk should be taken into account. As the focus of this study has been on predictive models, growth of *B. cereus* was quantitatively studied. Growth of *B. cereus* was shown to mainly take place during storage and cooling. Growth during storage was used as an example.

It was assumed that the temperature during storage is 6 ^{0}C, and pH and a_w of the product were assumed to be constant during the whole production process. The shelf life of the product was assumed to be 30 days (720 hours). Growth was predicted with the lag-exponential and the Baranyi models (Table 1), using the gamma model (Table 2) to estimate μ. The models result in minor differences in predicted growth (Fig. 3).

Figure 3 also shows growth estimates for T = 5 ^{0}C and T = 7 ^{0}C. The differences between the two primary models (Baranyi and lag-exponential) were negligible compared to the differences caused by the relatively small temperature variations (Fig. 3). It is very likely that the temperature can vary within the small range of 5 to 7 ^{0}C during storage. It was therefore decided that it was not useful to estimate and compare growth of *B. cereus* in cooked potatoes with more primary models. In this case it is best to use the simplest model available, which is the lag-exponential model.

Figure 4 shows the effects on the growth curve (predicted by the lag-exponential model) of using the gamma model, and a polynomial model (as used in the USDA Pathogen Modeling Program, version 5.0) for μ. In contrast to the primary models, the gamma and polynomial models result in a substantial difference in predicted growth at T = 6 ^{0}C. Also, small variations in temperature (T = 6 ^{0}C and T = 7 ^{0}C) did not rule out the substantial differences in model estimates for *B. cereus* in cooked potatoes. The same effects were observed for lag time (results not shown).

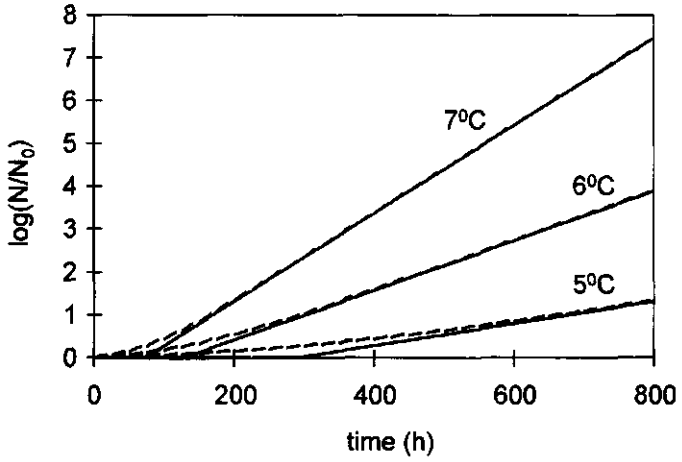

Fig. 3. Growth of *Bacillus cereus* at various temperatures (T = 5, 6, 7 °C), estimated by the lag-exponential model (——) and the Baranyi model (·····) (4).

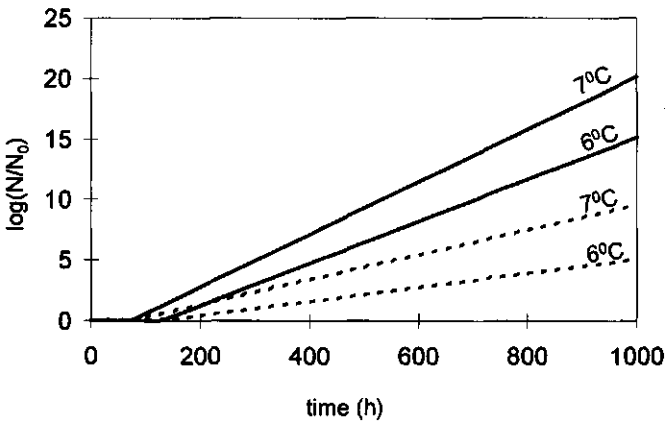

Fig. 4. Growth of psychrotrophic *Bacillus cereus* at various temperatures (T= 6,7 °C), predicted by the lag-exponential model. Growth rate was predicted by the USDA Pathogen Modeling Program (polynomial models) (——) and the gamma model (····).

The results, presented in Table 8, show that polynomial models for μ, and the gamma model for λ result in worst-case estimates in this example. However, the product was cooled at a temperature of 5 ^{0}C for 9 h before storage (Table 7); during cooling the *B. cereus* cells may have adapted to the low temperature. There may therefore not be a lag phase at all during storage. In this uncertain situation it is safest to choose $\lambda = 0$.

Using the lag-exponential model for the growth curve, polynomial models for μ, and assuming $\lambda = 0$ results in predictions that are not realistic: $\log(N/N_0) = 29$ for T= 6 ^{0}C. This high level will of course not be reached, but this quantitative prediction means that the organism may easily grow to the stationary phase. Using the gamma model for μ also resulted in a very high prediction of $\log(N/N_0)$: 9.2. In both situations the product is very unsafe. This would mean that the risk of becoming ill by consumption of the potatoes is very high if *B. cereus* is present. However, no foodborne outbreaks have been reported for vacuum packed cooked potatoes related to *B. cereus*. In practice, the probability of *B. cereus* contamination may be very low. Another likely reason for no reported outbreaks is that most of the bacteria present are vegetative cells (spores leftover after pasteurisation germinate, resulting in vegetative cells). These vegetative cells will generally be inactivated by a heat treatment before consumption, leading to non-infectious levels. This heat treatment by the consumer can easily be incorporated into the risk assessment by considering it to be part of the production process. The predictions of the models show clearly that growth of *B. cereus* after pasteurisation is likely. Experimental verification might be useful. It is shown that it can be useful to compare results of several predictive models in estimating risk. In contrast, it is also shown that process variations may affect risk more than the use of several models. In that case it is sensible to use the simplest model available.

Table 8. Specific growth rate (μ (h^{-1})) and lag time (λ (h)), estimated by the gamma model ($\lambda = 1/\mu$) and polynomial models (USDA Pathogen Modeling Program, version 5.0) at several temperatures

	μ_{gamma}	$\mu_{polynomial}$	λ_{gamma}	$\lambda_{polynomial}$
T=6 ^{0}C	0.013	0.044	75.2	411
T=7 ^{0}C	0.023	0.053	42.3	283

7. Conclusion

Many models describing growth and inactivation of microorganisms have been developed in past years. Growth and inactivation models that can be used in a stepwise procedure for quantitative risk assessments have been evaluated. The stepwise procedure uses three levels of detail, varying from rough to very detailed risk assessments, to first determine the most relevant phenomena before focusing on less relevant problems.

Risk-determining phenomena are found in the first level of detail by estimation of orders of magnitude for microbial processes. Orders of magnitude can efficiently be derived by using simple models.

Orders of magnitude for growth can be estimated by assuming first-order kinetics, neglecting lag phase and stationary growth. This generally results in worst-case estimates. Specific growth rate can easily be estimated by the gamma model, which has a simple structure and parameters that are available from the literature for many pathogens. Orders of magnitude for inactivation can be derived by assuming first-order kinetics as well. Shoulders and tailing phenomena are neglected, but this will generally not affect qualitative conclusions on the relevance of inactivation for risk. Values of inactivation rate can best be taken from literature. The models chosen for use in level 1 were the simplest models available, in order to find the main determinants of risk with the most efficiency.

In the second level of detail, the main determinants of risk are studied more accurately. In this level of detail, the lag-exponential and Baranyi model are applicable. For prediction of specific growth rate and lag phase, square root models were chosen because of practical advantages such as the availability and biological meaning of the parameters. For inactivation, the use of several models is restricted. Actually only the first-order inactivation model is generally applicable. Many primary and secondary models are not useful for predictive purposes, since parameter values are not available. After the above models, which were preferred on practical grounds, specific models may be chosen if appropriate for a certain case. As no model is able to accurately predict microbial responses under all circumstances, it is best to compare several models in quantitative risk assessment instead of relying on one model only.

Despite the benefits of assessing risk on a broad basis by comparing several model estimates, it was shown that comparison of models does not always substantially contribute to a broad view on risk. If process variations rule out differences between models, the accuracy of the model predictions do not justify the use of more com-

plex models. In those cases it is most efficient to use the simplest model available, an indication of the usefulness of simple models in advanced risk assessments.
If process variations do have a profound effect on risk it can be useful to take the frequency distributions of the process parameters into account in the most detailed level of risk assessment. The use of stochastic variables will however not change the conclusion on the usefulness of simple models in quantitative risk assessments.
The levels of detail provide an efficient way to first tackle the most relevant problems before focusing on less relevant problems. Efficiency in quantitative risk assessments has resulted from using simple models if there is no point in using complex models. This is for instance the case in estimating orders of magnitude by simple models to determine the main determinants of risk.
By the proposed structured approach, attention is only paid to those phenomena that are of relevance. For these phenomena more accurate point estimates or stochastic distributions can be determined. Furthermore, phenomena that are not quantitatively important are detected and can be omitted. This stepwise approach is necessary to efficiently assess risks in the complex context of food safety. If one begins by taking everything into account, one will presumably become lost in details.

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4

A data analysis of the irradiation parameter *D10* for bacteria and spores under various conditions

Abstract

This chapter provides approximate estimates for the irradiation parameter D_{10} to globally predict the effectiveness of any irradiation process. D_{10} is often reported to depend on many specific factors, implying that D_{10} cannot be estimated without exact knowledge of all factors involved. For specific questions these data can of course be useful but only if the conditions reported exactly match the specific question. Alternatively, this study determined the most relevant factors influencing D_{10}, by quantitatively analysing data from many references.

The best first step appeared to be a classification of the data into vegetative bacteria and spores. As expected, spores were found to have significantly higher D_{10} values (average 2.48 kGy) than vegetative bacteria (average 0.762 kGy). Further analyses of the vegetative bacteria confirmed the expected extreme irradiation resistance of non-pathogenic *Deinococcus radiodurans* (average 10.4 kGy). Furthermore the analysis identified *Enterococcus faecium, Alcaligenes* spp., and several members of the *Moraxella-Acinetobacter* group as having very high resistance at very low temperatures (average 3.65 kGy).

After exclusion of high- and low-resistance spores and some specific conditions showing relevant high or low D_{10} values, the average for spores was estimated to be 2.11 kGy. For vegetative bacteria this average was estimated to be 0.420 kGy. These approximate estimates are not definite, as they depend on the data used in the analyses. It is expected that inclusion of more data will not change the estimates to a great extent. The approximate estimates are therefore useful tools in designing and evaluating irradiation processes.

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1. Introduction

Gamma irradiation can be used as a method for preserving foods. Inactivation of microorganisms takes place by impairment of critical molecules or organelles, such as DNA and the cytoplasmic membrane (8,10,28,47).

Processing by irradiation, either alone or in combination with other treatments, offers some unique advantages over conventional methods. The advantages are (i) the opportunity to process foods after packaging, other than canning; (ii) the preservation of food in the fresh state for long periods with no noticeable loss; and (iii) the economic savings from the use of a low-energy, low-cost processing technique when compared to other food-processing methods, such as heat or refrigeration (47).

Recently, the U.S. Food and Drug Administration (FDA) approved the irradiation of fresh and frozen red meats such as beef, lamb, and pork for controlling disease-causing microorganisms. The FDA had previously approved irradiation for several other products. The European Commission also recently achieved a general agreement on irradiation of food products (11). These developments may lead toward more use of irradiation processes for food preservation in the future. The major drawback of irradiation processes is however consumer resistance.

Irradiation dose can be quantified by the term gray (Gy). A dose of 1 J/kg of absorbing material is equal to 1 Gy (32). The Codex Alimentarius Commission currently recommends 10 kGy as the upper dose level for irradiation processes (48). Inactivation of microorganisms by gamma irradiation can be quantitatively described by first-order kinetics, with the amount of surviving microorganisms (N) depending on the dose absorbed by the product (D). If N_0 is the initial number of organisms present, and D_{10} is the decimal reduction dose, then equation 1 quantitatively describes the first-order inactivation (10):

$$\log\frac{N}{N_0} = -\frac{1}{D_{10}}D \qquad (1)$$

Inactivation curves do not always show a straight line behaviour as equation 1 indicates. Sometimes a shoulder appears in the low-dose range before the linear slope begins, or a tail appears after the linear slope ends. In those cases D_{10} is often estimated by fitting a least-square regression line through the data points in such a way that the non-linear parts are excluded (6,8,13,14,25,43). Other models for quantitative description of irradiation inactivation have been described by Brynjolfsson (7) and Schmidt and Nank (36). All models use D_{10} to describe irradiation resistance. It was assumed that differences in D_{10} between the various methods can be neglected.

The value of D_{10} depends on several factors. According to Grecz *et al.* (15), the major differences that affect radiation resistance are as follows: (i) water content of the

cytoplasm, (ii) the size of target chromosomal DNA, (iii) the structure of the chromosomal DNA, and perhaps (iv) the multiplicity of genome material. As a rule, the simpler the life form, the more resistant it is to effects of ionising radiation. For instance, viruses are more resistant than bacteria, which are more resistant than moulds, which are more resistant than human beings (28).

In general, vegetative bacteria are less radiation resistant than spores. For example, the lower radiation resistance of *Bacillus cereus* vegetative cells compared to spores was confirmed by Thayer and Boyd (44). Several vegetative bacteria however, were reported to have similar or even higher resistance than spores; these include *Deinococcus radiodurans* (2) and the *Moraxella-Acinetobacter* (M-A) group (12,25,46). Also, *Enterococcus faecium* and members of the group *Achromobacter-Alcaligenes* (A-A) might, in the frozen state, acquire resistance comparable to that of *Clostridium botulinum* spores (26). The difference between the M-A and the A-A group is not always clear (46).

Bacteria species of the same genus have been shown to have different irradiation resistance (6,33,41). Moreover, several studies reported that strains belonging to the same species may not have similar resistance (3,4,13,32). Nevertheless, the general assumption is that bacteria of the same species are closely related in several properties including irradiation resistance (15).

Some organisms appear to be more susceptible to irradiation at low doses when irradiated during the exponential phase of growth than during the stationary phase, as observed for *Listeria monocytogenes* (20), *B. cereus* vegetative cells (44), *Escherichia coli* O157:H7 (43), and *Staphylococcus aureus* (27). *Aeromonas hydrophila* (29) and *Campylobacter jejuni* (24), however, had similar values for stationary and exponential growth phases.

Gram-negative bacteria are generally more sensitive to irradiation than Gram-positive bacteria (2,5,19).

Many studies were performed to estimate the influence of the irradiation medium on D_{10}, e.g., low-fat versus high-fat media, dry versus aqueous media, frozen versus unfrozen media, and media with different values for water activity. The results of these studies were not always similar and were frequently contradictory. For example, Diehl (10) cited a study that reported the protective effect of low-fat ground beef toward *S. aureus*, whereas Monk *et al.* (27) showed no significant differences in D_{10} of *S. aureus* in low-fat and high-fat ground beef.

Also, many studies were performed to estimate temperature effects during irradiation. A protective effect of decreasing temperatures is generally assumed, which may be due mainly to the decreased mobility of free radicals (2,25). The radiation sensi-

tivity of vegetative bacteria was reported to be reduced by a factor 2 to 5 when irradiation takes place in the frozen state as compared with room temperature (26). Such results have been reported for various vegetative bacteria. The radiosensitivity of spores was reported to vary notably less or not at all (26). The resistance of strain 33A *C. botulinum* spores was reported to rise linearly in beef as the radiation temperature was lowered (2).

The presence of oxygen during irradiation has been found to enhance the lethal effect of irradiation due to oxygen radical formation (39). This was, for example, confirmed for several strains of *Salmonella typhimurium* (13,42), *Yersinia enterocolitica* (13), and *L. monocytogenes* (13). In contrast, several studies found irradiation resistance to be unaffected by air (43,44) or even to be decreased in atmospheres from which oxygen was excluded (30).

Some other factors that have also been studied in relation to irradiation resistance are, for example, the initial cell concentration (1,9,17), recovery medium (12,13,31,41), preheating (25,37), and pre-irradiation (26,46).

As shown, the literature contains many specific studies on factors influencing D_{10}. The quantitative influence of these factors is not known. Yet, it is important to know which factors are quantitatively relevant and to estimate unknown D_{10} values under specific conditions. By estimating unknown D_{10} values, global predictions on the effectiveness of any irradiation process can be made. The purpose of this study was therefore to estimate quantitatively the influence of various factors on D_{10}. Based on many specific D_{10} values from the literature, general quantitative conclusions on D_{10} in relation to environmental factors were drawn.

2. Materials and Methods

A total of 539 D_{10} values was gathered from 40 references. Most D_{10} values had been estimated by linear regression, although some references had used the formula of Schmidt and Nank (3,4,16,17,37).

2.1 Analysis of variance

Various references reported D_{10} values with related variances. The condition of equal variances was tested by linear regression. In addition, residual plots were studied to find which data transformation: none, a square root one, or a logarithmic one (natural logarithm) was necessary to stabilise variances (49).

2.2 Stepwise data analysis

For data analysis, the total amount of sorted data was divided into three equally large categories. This allows fair comparisons between the categories, and extreme high or low data do not substantially affect the boundaries between the categories. The data at and above the upper boundary, and at and below the lower boundary were studied for trends. A trend in the data was assumed if the majority (≥ 50%) of a group of data is situated in one of the categories. Trends indicate factors related to high or low resistance. A trend may totally depend on data from one reference. In such cases the validity of the trend can sometimes be questioned. For example: if, in case of a high trend for a factor *X*, most other data in the specific reference are also above the upper boundary, the high trend may have been caused by a structural error or other specific experimental condition. Such cases are mentioned in the text. Groups with only two or fewer data points were not taken into account.

Additionally, multivariate analysis (MVA) was performed to estimate factors having quantitative influence on irradiation resistance. For this purpose a model is fitted to the data, describing irradiation resistance as the response variable of all factors. The MVAs were performed in SAS, release 6.12 (the SAS Institute Inc., Cary, N. C.). The results of MVA were compared to the results of the trends analysis. If MVA confirmed high or low trends, the trends were assumed to be valid.

In the first instance, we determined whether trends were associated with the following factors: microbial identity (groups: vegetative bacteria and spore-forming bacteria), medium (groups: products and broth), temperature (groups: frozen and not frozen), Gram stain (groups: Gram negative and Gram positive), and atmosphere (groups: aerobic and anaerobic). In further studies, the most relevant groups were analysed in more detail, and the influence of temperature (numeric value) was studied by linear regression. Based on these analyses further divisions can be made, if necessary.

3. Results

3.1 Analysis of variance

Data transformation was shown to be necessary to stabilise variances, because the reported variances appeared to increase with increasing D_{10} (slope = 0.00472; t = 7.69; $t_{critical}$ = 1.98 at 106 df and a 95% confidence interval). The square root and

Table 1. Data analysis of 539 data on radiation resistance

Factor	microbial identity		medium		temperature		atmosphere		Gram stain	
Group	vegetative	spores	products	broth	frozen ($\leq 0^0$C)	non frozen ($> 0^0$C)	anaerobic	aerobic	Gram negative	Gram positive
# data *	360	179	326	213	139	332	185	254	225	314
above upper boundary (%) †	8.06	83.8	25.2	45.5	41.0	27.4	38.4	19.3	5.33	53.2
below lower boundary (%) †	49.7	0	35.3	30.0	23.7	38.0	25.9	43.7	60.9	13.4
Tr ‡	0.627		0.128		0.140		0.185		0.477	
RSS_{total} §	737.2		737.2		572.2		504.9		737.2	
RSS_{factor} §	400.5		715.2		533.2		475.6		539.3	
profit §	336.7		22		39		29.3		197.9	

* Several data were reported without explicitely mentioning all factors. For this reason, the sum of data for a factor is not always equal to 539.

† The lower and upper boundary were respectively based on the 180th and 360st value for $\ln(D_{10})$; given that the $\ln(D_{10})$ values were sorted in ascending order.

‡ Tr is a characteristic number to compare the relevance of a trend for one group compared to another group, and is given by $Tr = |(X_a - Y_a) + (Y_b - X_b)|/200$. Tr varies between 0 (no trend) and 1 (very clear trend). $X_{a,b}$ and $Y_{a,b}$ denote percentages of data of the groups X and Y. The subscript a means above the upper boundary, b means below the lower boundary.

§ profit = RSS_{total} - RSS_{factor} with RSS_{total} is the residual sum of squares (RSS) resulting from quantitative description of all $\ln(D_{10})$ as the average $\ln(D_{10})$, RSS_{factor} is the RSS resulting from description of $\ln(D_{10})$ according to the specified factor.

logarithmic transformation resulted in non-significant correlation (slope = $1.50*10^{-5}$; t = 0.0291, and slope = 0.00330; t = -1.53, respectively). Residual plots showed the logarithmic transformation to be a better data transformation than the square root transformation, because the logarithmic data transformation resulted in a more even spread of the residuals around 0.

3.2 Stepwise analysis of data

The 539 data (average $\ln(D_{10})$ = -0.368) were divided into three categories, containing 180, 180, and 179 data, respectively. Trends were studied for the factors: identity of microorganism, medium, atmosphere, Gram stain, and temperature. The results are shown in Table 1. It appeared that clear trends were visible with respect to microbial identity and Gram stain. The characteristic number *Tr* (Table 1) confirms this. *Tr* is highest for microbial identity, *Tr* = 0.627 (Table 1), indicating that the trend for this factor is clearest. All spore formers were Gram positive, and the majority of vegetative cells were Gram negative bacteria. For this reason it is sensible that the existence of clear trends for both microbial identity and the factor Gram stain is related to the high correlation between the factors microbial identity and Gram stain. Analysis of variance (single factor) showed identity of microorganisms to describe $\ln(D_{10})$ better than the other factors; the decrease in the residual sum of squares (RSS) was largest when the classification of vegetative bacteria and spores was used for description of $\ln(D_{10})$ (see profit, Table 1).

For all the above reasons the best first step is to divide the 539 data into vegetative bacteria (average $\ln(D_{10})$ = -0.925) and spores (average $\ln(D_{10})$ = 0.753) . It is remarkable that the 8% of the data for vegetative cells that were above the upper boundary were amongst the highest reported $\ln(D_{10})$ values (Fig. 1).

3.3 Spores

As in the first step, the 179 data for spores were divided again into three categories, containing 60, 60 and 59 data, respectively. Further data analyses were performed for the factors microbial identity, medium, and atmosphere.

The factor microbial identity concerned the following organisms: *B. cereus, B. licheniformis, B. megaterium, B. pantothenticus, B. pumilus, B. stearothermophilus, B. subtilis, C. aerofoetidum, C. bifermentans, C. botulinum, C. butyricum, C. colinitolerans, C. chauvoei, C. fallax, C. histolyticum, C. oedematiens, C. perfringens, C. septicum, C. sordellii, C. sphenoides, C. sporogenes, C. subterminale, C. tetani, C. tertium, C. tetanomorphum,* and *Sporolactobacillus inulinus.*

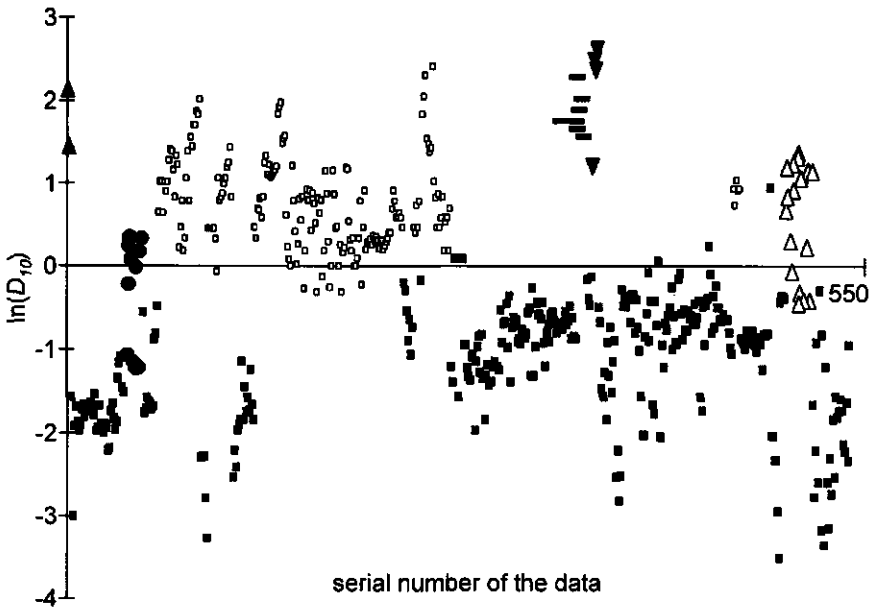

Fig. 1. Reported ln(D_{10}) values in arbitrary order. ▲ are ln(D_{10}) for *Acinetobacter calcoaceticus*; ● are ln(D_{10}) for *Alcaligenes spp.*; ▼ are ln(D_{10}) for *Deinococcus radiodurans;* - are the ln(D_{10}) values for various *Moraxella*; Δ are ln(D_{10}) for *Enterococcus faecium.* ■ are ln(D_{10}) values for other vegetative cells. □ are ln(D_{10}) values for spores.

The factor medium consisted of the following media: animal fat, beef, beef stew, broth, chopped ham, codfish cake, corned beef, dairy, distilled water, glucose, glycerol, lyophilised broth, mechanically deboned chicken, vegetable oil, phosphate buffer, plastic material, pork loin, pork sausage, salt solution, spore suspension, tributyrin, triolein, and turkey breast.

The factor atmosphere consisted of: air, vacuum, N_2

All spores were Gram positive bacteria, so this factor was not considered. The influence of temperature was studied quantitatively by linear regression, showing that temperature does significantly influence ln(D_{10}) (slope = -0.00336; t = - 4.37; $t_{critical}$ = -1.98). If the 5 ln(D_{10}) values for T= -196 ^{0}C (16) were excluded (generally not practically relevant), no significant correlation between temperature and ln(D_{10}) was found (slope = $-3.6*10^{-4}$; t = -0.228; $t_{critical}$ = -1.98). MVA also showed that temperature does not significantly contribute to a better quantitative description of

$\ln(D_{10})$, so temperature was not considered to influence quantitatively $\ln(D_{10})$ for spores in the relevant temperature range. Several MVA were performed, to determine quantitatively the influences on $\ln(D_{10})$. For spores, these analyses were on the factors: microbial identity (MI), medium (ME), MI+ME, MI+ME+MI·ME, MI+ME+atmosphere (AT). As an example, the results of the variate analyses on the factors MI, and MI+ME are shown below. Only coefficients significantly different from 0 (95% confidence level), and based on more than 2 $\ln(D_{10})$ values are shown.

factor MI: $\ln(D_{10}) = 0.9155 + 0.6997X_1 + 0.5507X_2$

with X_1 = dummy variable (0 or 1) for *B. stearothermophilus;* X_2 = dummy variable for *C. sporogenes. S. inulinus* was the reference spore in this analysis using dummy variables, meaning that *S. inulinus* was set at zero, whereas the other factors were set at one. The results show that only *B. stearothermophilus* and *C. sporogenes* are significantly different from, and result in, higher $\ln(D_{10})$ values than *S. inulinus.*

factor MI+ME:

$$\ln(D_{10}) = 0.7222 + 0.6085X_1 + 0.6930X_2 + 0.6049X_3 + 0.5151X_4 + 0.8317Y_1 + 0.6853Y_2 - 0.7163Y_3 - 0.4820Y_4 + 0.9082Y_5 + 0.6243Y_6 - 0.9429Y_7 - 0.4408Y_8 - 0.7606Y_9$$

with X_1 = dummy variable for *B. stearothermophilus;* X_2 = dummy variable for *C. histolyticum;* X_3 = dummy variable for *C. oedematiens;* X_4 = dummy variable for *C. sporogenes. S. inulinus* was the reference spore in this analysis using dummy variables. Y_1 = dummy variable for animal fats; Y_2 = dummy variable for beef; Y_3 = dummy variable for beef stew; Y_4 = dummy variable for corned beef; Y_5 = dummy variable for dairy; Y_6 = dummy variable for glycerol; Y_7 = dummy variable for pork sausage; Y_8 = dummy variable for salt solution; Y_9 = dummy variable for spore suspension. Water was the reference product in this analysis using dummy variables.
A trend was considered to be confirmed if two or more of the above MVA showed significantly high or low $\ln(D_{10})$ values for the specific factor.
Table 2 shows the results of the trends analysis for spores for the factors MI, ME, and AT. *C. sporogenes* and *B. stearothermophilus* showed high trends that were confirmed by MVA. The animal fat data were all measured for *C. sporogenes.* The high trend may thus be due to the organism instead of the product. MVA however clearly confirmed the high $\ln(D_{10})$ values for animal fat compared to other products, and therefore animal fat was also assumed to cause a high trend. The high trends for

Table 2. Organisms, media, atmospheres, and growth phases causing high or low trends. Bold data indicate factors with high or low trends that were confirmed by MVA

Spores			
HIGH TREND	% aub *	LOW TREND	% blb †
Organisms			
Bacillus cereus	62	*C. botulinum*	50
B. stearothermophilus	87		
Clostridium sporogenes	75		
Sporolactobacillus inulinus	60		
Media			
glucose	70	**pork sausage**	80
phosphate buffer	69	**beef stew**	100
glycerol	100	**corned beef**	50
animal fats	100		
dairy	100		
Atmosphere			
N_2	100		
Vegetative bacteria			
Organisms			
***Alcaligenes* spp.**	58	***Aeromonas hydrophila***	94
***C. perfringens* (vegetative cells)**	57	*B. cereus* (vegetative cells)	63
Moraxella osloensis	100	*B. subtilis* (vegetative cells)	100
M. phenylpyruvica	100	***Campylobacter jejuni***	87
Deinococcus radiodurans	100	***Pseudomonas putida***	100
Enterococcus faecalis	100	*S. gallinarum*	50
E. faecium	100	***Yersinia enterocolitica***	93
***Salmonella* spp.**	89		
S. typhimurium	62		
Media			
roast beef	57	ground bluefish	87
cauliflower	57	**filet americain**	60
lyophilised broth	50	trypticase soy broth	100
eggs	50		
horse meat	100		
Atmosphere			
N_2	57		
Growth phase			
		exponential growth phase	63

* % aub is the percentage of the data of a specific group above the upper boundary.
† % blb is the percentage of the data of a specific group below the lower boundary.

B. cereus and *S. inulinus* were not confirmed by MVA, so they were not assumed to be relevant. The high trends for glycerol and dairy were confirmed by MVA and were therefore assumed to be relevant, in contrast to the trends for phosphate buffer and glucose that were not confirmed by MVA. The high trend for N_2 was confirmed by MVA. The data were from one reference only (16), and the other data in this reference were all above the upper boundary as well. This indicates that the high values for N_2 may be due to the specific reference. The values for N_2 are however much higher than other $\ln(D_{10})$ values, and therefore the group was taken separately as a specific high $\ln(D_{10})$ effect.

The low trend for *C. botulinum* was not confirmed by MVA, and therefore it was not assumed to be relevant. The low trends for the products pork sausage, corned beef, and beef stew were confirmed by MVA. The data for beef stew were taken from one reference only (37), containing no other data. The data for pork sausage and corned beef were also from one reference (3). This reference does however also contain data for other media, not resulting in low trends. The trends for the products were all assumed to be relevant.

Based on the results of the data analyses the following classification was made: high-resistance situations: average $\ln(D_{10})$ = 1.48 kGy (*B. stearothermophilus, C. sporogenes,* glycerol, animal fats, dairy); N_2: average $\ln(D_{10})$ = 1.72 kGy; low-resistance situations: average $\ln(D_{10})$ = 0.286 kGy (pork sausage, beef stew, corned beef); and all other conditions: average $\ln(D_{10})$ = 0.681 kGy. The averages of high-resistance situations and N_2 were not significantly different, and therefore the groups were combined, resulting in an average $\ln(D_{10})$ = 1.53 kGy. Fig. 2 shows the classification of the data with the average D_{10} values (untransformed data) for each group.

3.4 Vegetative bacteria

The 360 data for vegetative bacteria were analysed in the same way as the data for spores. Further data analyses were performed for the factors MI, ME, and AT.

The factor MI concerned the following organisms: *Acinetobacter calcoaceticus, A. hydrophila, Alcaligenes, B. cereus* (vegetative cells), *B. subtilis* (vegetative cells), *C. jejuni, C. perfringens* (vegetative cells), *C. sporogenes* (vegetative cells), *E. coli, Lactobacillus spp., L. innocua, L. ivanovii, L. monocytogenes, Moraxella nonliquefaciens, M. osloensis, M. phenylpyruvica, D. radiodurans, Pseudomonas, P. putida, Salmonella* spp., *S. anatum, S. enteritidus, S. gallinarum, S. meleagridis, S. panama, S. schottmuelleri, S. senftenberg, S. stanley, S. typhimurium, S. inulinus* (vegetative cells), *S. aureus, E. faecalis, E. faecium, Vibrio alginolyticus, V. cholerae, V. fluvialis, V. mimicus, V. parahaemolyticus, V. vulnificus,* and *Y. enterocolitica.*

The factor ME consisted of the following media: beef, broth, bone meal, cauliflower, egg, filet americain, glucose, gravy, ground beef, ground bluefish, ground pork, ground turkey, horse meat, lamb, lyophilised broth, mechanically deboned chicken, minced chicken, minced pork, phosphate buffer, pork, potato, prawn, roast beef, roast potato, salt solution, shrimp, and turkey.
The factor AT consisted of air, vacuum, microaerobic, N_2, CO_2:N_2, and CO_2.
The Gram stain and growth phase (exponential and stationary growth phase) were also studied as possible influencing factors. The results are shown in Table 2.
The influence of temperature was studied quantitatively by linear regression, showing that temperature does significantly influence $\ln(D_{10})$ for vegetative bacteria: the higher the temperature, the lower $\ln(D_{10})$ (slope = -0.0159, for -196 ^{0}C to 55 ^{0}C, t = -10.0, $t_{critical}$ = -1.98). Even if extravagant data (-196 ^{0}C) were removed, there was still a significantly relevant slope. Several (multi)variate analyses were performed; for vegetative bacteria these analyses were on the factors: MI, ME, MI+ME, MI+ME+temperature (TM), MI+ME+MI·TM, and MI+ME+MI·TM+AT.
The high trends for *Alcaligenes* spp., *C. perfringens* (vegetative cells), *D. radiodurans, E. faecalis, E. faecium, M. osloensis* (one ref. (46) with all other data above the upper boundary as well), *M. phenylpyruvica, Salmonella* spp., and *S. typhimurium* were all confirmed by MVA, as well as the high trends for lyophilised broth, horse meat, and eggs. The high trends for roast beef and cauliflower were not confirmed by MVA. The high trend for N_2 was again, as for spores, confirmed by MVA.
It is remarkable that all data for *D. radiodurans* (25) and *M. osloensis* (46), and the majority of the data for *E. faecium* (2,26) were far above the other data for vegetative bacteria (Fig. 1; also shown by MVA). As its name indicates, *D. radiodurans* is very irradiation resistant. For *M. osloensis* and *E. faecium* these high $\ln(D_{10})$ values were measured at temperatures ≤ -30 ^{0}C. The few high $\ln(D_{10})$ values for *A. calcoaceticus* (46) and *Alcaligenes* spp. (26) (Fig. 1) were also measured at these temperatures. This indicates that temperature may have caused the extremely high values for these organisms. Other organisms such as *Pseudomonas, L. monocytogenes, E. coli,* and *C. jejuni*, however, did not show these extremely high $\ln(D_{10})$ values at very low temperatures (1,12,21,25,26). It was assumed that the combination of high cell resistance and temperature caused the very high $\ln(D_{10})$ values. This assumption could, however, not be confirmed by MVA, because there were not enough data per organism at various temperatures. The low trends for *A. hydrophila, C. jejuni, P. putida,* and *Y. enterocolitica* were confirmed by MVA, as well as the low trend for filet americain.

Based on the analyses, a division was made in the following rank order (Fig. 2): *D. radiodurans*: average $\ln(D_{10})$ = 2.22 kGy; very high-resistance situations: average $\ln(D_{10})$ = 1.05 kGy (*Alcaligenes, Acinetobacter, E. faecium,* and *M. osloensis* at very low temperatures (≤ -30 ^{0}C)); high-resistance situations: average $\ln(D_{10})$ = -0.611 kGy (*Alcaligenes* spp., *C. perfringens* vegetative cells, *E. faecalis, E. faecium, M. phenylpyruvica, Salmonella* spp., *S. typhimurium,* N_2, horse meat, lyophilised broth, eggs); low-resistance situations: average $\ln(D_{10})$ = -1.85 kGy (*A. hydrophila, C. jejuni, P. putida, Y. enterocolitica,* and filet americain); and all other conditions: average $\ln(D_{10})$ = -1.04 kGy.

4. Discussion

To estimate quantitatively the influence of various factors on the irradiation parameter D_{10}, many data from numerous references were analysed.

Spores of *B. stearothermophilus* and *C. sporogenes* were shown to be highly resistant. This is a remarkable conclusion, since the apparent high irradiation resistance was not explicitly mentioned in the literature before. Based on personal measurements, Briggs (6) concluded that *B. stearothermophilus* spores are among the high radiation (and heat)-resistant aerobic spores. For *C. sporogenes* it has been established that it is not substantially more resistant than *C. botulinum* types A and B (4,35). Both references (4,35), however, noted the high resistance of *C. sporogenes* PA 3679. This explains the conclusion in this study of *C. sporogenes* spores being highly resistant, as it was based on data for PA 3679.

The high D_{10} values measured in media with glycerol and animal fats may be explained by a protective effect resulting from decreased water activity. The capability of glycerol as a scavenger for toxic radiolysis products of water was mentioned in the literature (18,38). This is consistent with the high resistance in media with glycerol. A scavenging effect of proteins was mentioned by Diehl (10). This scavenging effect of proteins may explain the high resistance in dairy products, generally containing high levels of proteins. N_2 apparently resulted in high D_{10} values for spores, as well as for vegetative bacteria. As mentioned by Stapleton *et al.* (39) and Diehl (10), this could be explained by the absence of oxygen radical formation.

In conclusion, it is important to realise that high-resistance spores and conditions promoting high resistance exist in irradiation processes. If it is suspected that these or similar situations that may result in high resistance apply in irradiation processes,

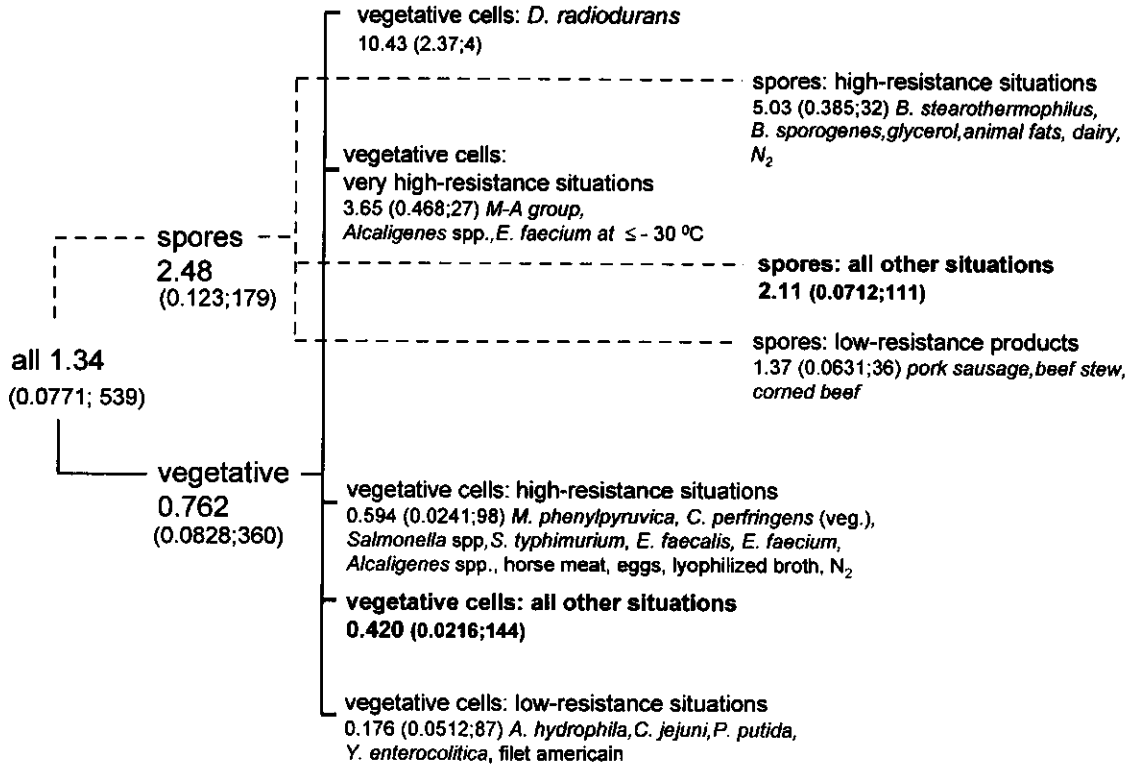

Fig. 2. Classification of all D_{10} data into groups with the average D_{10} value of the group. M-A group: *Moraxella-Acinetobacter* group. Between brackets are the standard deviations of the average D_{10}, and the number of data respectively.

it is important to take proper (extra) control measures; irradiation at 10 kGy will result in a 10^2 reduction only for the high-resistance spores, and under conditions showing relevant high D_{10} values. Generally, under normal conditions, reduction of spores at 10 kGy is about 10^5 based on an average of 2.11 kGy (Fig. 2).

Various vegetative bacteria were shown to be highly resistant. This study confirmed the very high resistances of *D. radiodurans*, *E. faecium*, *Alcaligenes* spp., and members of the *Moraxella-Acinetobacter* (M-A) group that were frequently mentioned in the literature (2,12,25,26,46). *D. radiodurans* showed very high resistance under various conditions. *D. radiodurans* appears however not to be relevant for food, as it is neither a food spoilage organism, a public health hazard, nor a measure of food sanitation. *E. faecium*, *Alcaligenes*, *Acinetobacter*, and *M. osloensis* showed very high D_{10} values at low temperatures (≤ -30 ^{0}C). A sudden increase in radiation resistance between 0 and -20 ^{0}C was mentioned before (16), and was assumed to correlate possibly with the solidification of water in the cell within this critical temperature range. This, and the fact that temperature was shown to be inversely proportional to $\ln(D_{10})$ may explain the high resistance of these organisms under these conditions. *E. faecium*, *Alcaligenes* spp, and *A. calcoaceticus* may be pathogenic to humans, but they were not conclusively proven to be foodborne (22,23,34,45). Irradiation at 10 kGy results in a 10^2 to 10^3 reduction only, and this is generally not enough with respect to food safety. If these are the target organisms a combination of treatments may be necessary to ensure food safety.

Salmonella spp., *C. perfringens* vegetative cells, and *E. faecalis* were shown to be among the high-resistance vegetative bacteria. This is in agreement with conclusions drawn from the literature (8,13,30,40). The high $\ln(D_{10})$ values for lyophilised broth are probably due to decreased water activity, and the high resistance related to eggs may be explained by the high protein content of this product. There is no obvious reason for the high $\ln(D_{10})$ values with horse meat. The values were measured at -17 ^{0}C, which partly explains the high resistance. Again, it is important to realise that highly resistant bacteria exist, and that conditions may confer to bacteria increased resistance to irradiation. This is especially relevant for irradiation processes at much less than 10 kGy; at 10 kGy, reduction is very high anyway for these organisms. During irradiation processes at 10 kGy vegetative bacteria are generally reduced by $> 10^{20}$ based on the averages of 0.594 kGy for high-resistance conditions and of 0.420 for other situations (Fig. 2), showing no practical reasons to determine if high resistance situations exist or not.

If unknown D_{10} are to be estimated, it is important to consider whether the conditions may confer high resistance or very high resistance, for example, if D_{10} for

enterococci have to be estimated at room temperature, it is sensible to use the high-resistance situations (Fig. 2) estimation, instead of the very high-resistance situations estimation. In case of double options or doubt, it is sensible to choose the worst-case scenario, for example, the estimation of D_{10} for the highly resistant spores of *B. stearothermophilus* in the low resistance product beef stew would be 5.03 kGy (Fig. 2).

In the field of predictive microbiology, many models for growth and, to a lesser extent, for thermal inactivation of microorganisms have been developed. It was not the purpose of this study to develop a predictive model for irradiation inactivation. Its purpose was to estimate quantitatively the most relevant determinants of the irradiation parameter D_{10}. It is interesting to model D_{10} quantitatively in relation to environmental factors, as applications of irradiation processes may be extended.

This study used stepwise trend analysis supplemented by MVA. It was shown that the methods did not give definite answers to the complex problem, for example, apparent trends were not always confirmed by MVA, and MVA gave different results at various analyses. This is not surprising, because the meta-analysis consisted of a very high variety of qualitative factors. Moreover these factors may (interactively) influence D_{10}. It was therefore sensible not to rely on one method; instead, the advantages of the non-conventional semi-quantitative trends analysis were combined with the advantages of the conventional quantitative MVA to find important factors influencing D_{10}.

This chapter provides approximate estimates for D_{10} under various conditions. The estimates are based on 539 data from the literature for various organisms, media, atmospheres etc. The classification shown in Fig. 2 should not be considered as definite. It is however not expected that the classification will change much as a result of adding data, because we used many data from many references. For this reason, the approximate estimates provided are useful tools in evaluating irradiation processes.

5. Conclusion

This chapter presents a rough classification of microorganisms and irradiation conditions to bring about D_{10} categories to estimate globally the effectiveness of any irradiation process. This chapter shows that sensible approximate estimates are possible without exact knowledge of all factors involved.

The difference between spores and vegetative cells was shown to be the greatest factor influencing the magnitude of D_{10} values, spores generally being more radia-

tion resistant than vegetative cells. There are however several vegetative microorganisms for which extreme high resistance was found. Some of these (*E. faecium, Alcaligenes* spp., and *A. calcoaceticus*, at temperatures below -30 ^{0}C) may be pathogenic to humans. In conclusion, irradiation processes can be used to significantly reduce dangerous food-related microorganisms such as *C. jejuni, Salmonella* spp., *L. monocytogenes,* and *E. coli* O157:H7. A combination treatment may however be necessary to control food safety, specially targeted at the high radiation-resistant microorganisms.

The effectiveness of irradiation processes for food safety and for prevention of food spoilage depends on many factors, as do practically all microbial processes in food products. When developing (new) food processes it is impossible to take every factor into account. The approximate estimates for D_{10} are based on the most relevant factors for irradiation, and therefore can serve as a useful guide when designing safe food processes.

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5

Stepwise quantitative risk assessment as a tool for characterisation of microbiological food safety

Abstract

This chapter describes a general method for microbial quantitative risk assessment for food products and their production processes. The method applies stepwise quantitative risk assessment, allowing to tackle first the main problems before focusing on less relevant problems. Firstly, risks are assessed broadly, using order of magnitude estimates. Variations in process or product parameters can easily be evaluated at this level. Characteristic numbers are used to quantitatively characterise microbial behaviour during the production process. These numbers help to highlight the risk-determining phenomena, and to find negligible aspects. Secondly, the risk-determining phenomena are studied more accurately. Both general and/or specific models, and various scenarios can be used to quantitatively describe the risk-determining phenomena, providing a broad view on risk. Thirdly, even more accurate studies can be performed where necessary, by using for instance stochastic variables. All steps of the method are transparent, and therefore every step can easily be criticised.

The method for quantitative risk assessment has been implemented as a decision supporting expert system; the SIEFE model: Stepwise and Interactive Evaluation of Food safety by an Expert System. The SIEFE model provides a tool for bacterial risk assessments in a structured manner, using various knowledge sources. The main goal of the SIEFE model is giving quantitative insight into microbial behaviour during production processes, and thereby serve as a tool for decision making.

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1. Introduction

Food safety is important for consumers, food producers and inspection authorities for numerous reasons, for example consumer protection, producers' risk, and international trade. Food safety management systems, such as HACCP, are often applied in a qualitative way. These systems can be supplemented with quantitative risk assessment to study food production processes quantitatively. Quantitative risk assessment provides improved understanding of factors involved in food safety. This means that problems regarding food safety can effectively be prevented, and that necessity and effects of control measures can be assessed quantitatively. Processes can be optimised according to a certain risk, and safety criteria can be based on quantitative risk assessment.

In literature, several quantitative risk assessments for specific microbiological hazards in products have been described. For example for *Listeria monocytogenes* in bovine milk (32) and soft cheese (5), *Salmonella enteritidis* in pasteurised liquid eggs (47), *Salmonella* in cracked eggs (41), *Salmonella* in chicken products (7,31), *Escherichia coli* O157:H7 in hamburgers (9,24), *Bacillus cereus* in pasteurised milk (30), and *Taenia saginata* in cattle (43).

In contrast to these quantitative risk assessments for specific food products, McNab (27) presented an approach for quantitative risk assessment for microbial food safety in general. The present study also describes a method for systematic quantitative risk assessment for microbial safety of food products. The method is stepwise and interactive, and has been developed for bacteria. The method has been implemented as a decision supporting expert system; the SIEFE model: Stepwise and Interactive Evaluation of Food safety by an Expert system.

2. The SIEFE model as a structured method for quantitative risk assessment

The SIEFE model has been developed for stepwise and interactive quantitative risk assessment associated with microbial hazards for food products and production processes. The stepwise approach uses three levels of detail, ranging from semi-quantitative, rough risk assessments to detailed quantitative risk assessments. Its structured way of assessing risks may provide new insights into production processes, and helps preventing important aspects from being overlooked. The general framework of the procedure is shown in Figure 1.

The SIEFE model starts with hazard identification. Hazards can be identified at three levels of detail according to the hazard identification procedure proposed by Van

Gerwen *et al.* (45). The level of detail in the hazard identification procedure does not have to be the same as the level of detail in the following steps of the SIEFE model. Actually, the hazard identification procedure can be seen as a stand alone part of the SIEFE model (Fig. 2). The first and second level of the other parts of the SIEFE model are described in the next sections.

3. Level 1 risk assessment

The first level of detail is a rough, semi-quantitative level. This level provides rough estimates of risks related to the consumption of food products and shows risk-determining aspects.

3.1 Level 1: Exposure assessment - process identification

At the first level of detail, process steps and related data for time, temperature (T), pH, and water activity (a_w) are gathered, and entered in a table as in a spreadsheet. Temperature, pH, and a_w are assumed to remain constant during a process step. The estimated orders of magnitude in the first level of detail are generally on the safe side to be able to omit quantitatively negligible aspects without missing any potential relevant ones. For certain process steps the estimates might overestimate the relevance for risk. This will then be detected in level 2.

3.2 Level 1: Exposure assessment - contamination

In the first level of detail it is assumed that all products are contaminated. Initial contamination level is assumed to be one per serving, so N_0 = 1 CFU·serving^{-1}. A serving is assumed to generally contain 100 g of the product. As a consequence, exposure is actually based on the change of the concentration of organisms in a serving, instead of being estimated as a concentration of organisms present. Knowledge rules highlight the necessity of changing this assumption in cases where contamination level may greatly influence risk.

3.3 Level 1: Exposure assessment - growth & inactivation

At level 1, orders of magnitude for inactivation and growth are estimated by first order kinetics. The logarithm of the increase or decrease of microorganisms can then be estimated by: $\ln\left(\frac{N}{N_0}\right) = vt$, with N as the concentration of organisms (CFU·serving^{-1}), and N_0 as the initial concentration of organisms per serving.

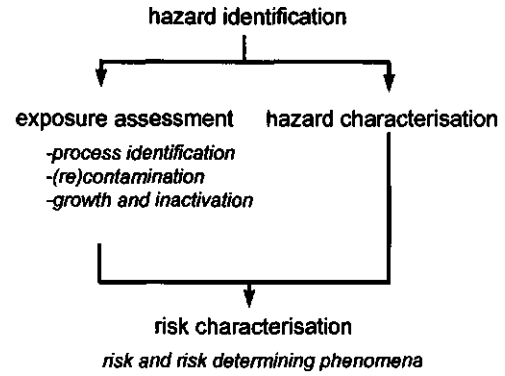

Fig. 1. Framework of the procedure for quantitative risk assessment.

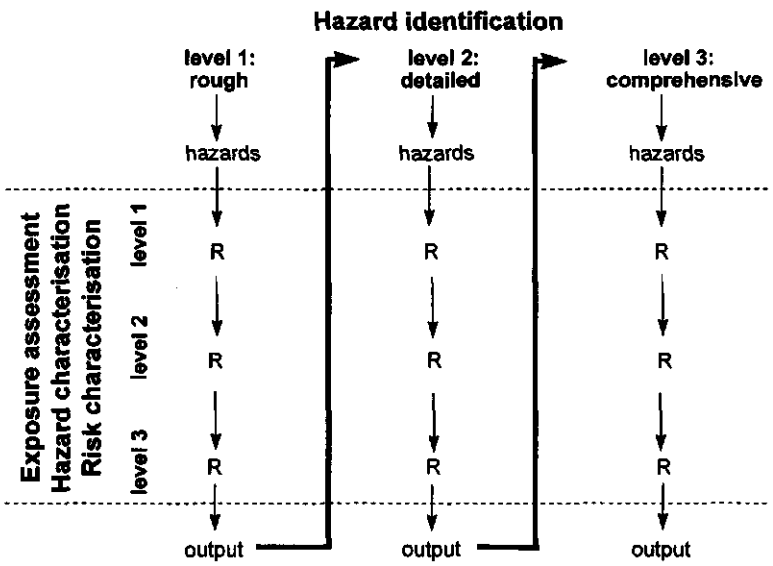

Fig. 2. The levels of detail of the hazard identication procedure (Van Gerwen *et al.*, 1997) in relation to the levels of detail of the other parts of the SIEFE model. R means risk estimate & risk determining phenomena. The output consists of insight into the risk assessment and the food production process under study. This output is used to decide on further steps to be taken.

For growth, $v = \mu$. The value for the maximum specific growth rate μ (s^{-1}) is estimated by means of the gamma model (48). The necessary growth characteristics were derived from the literature for various pathogens, and placed into the pathogen database (45). Lag time is neglected in level 1, which results in fail safe predictions. For inactivation, $v = -k$. The SIEFE model estimates the value for the inactivation rate k (s^{-1}) from D values (D), with:

$$k = \ln(10)/D \qquad (1)$$

D values were reported for many pathogens under various conditions in the literature. These data from the literature for various pathogens were also included in the pathogen database. For a selected hazard, the expert system takes data from the database and describes $\log(D)$ as a response variable of temperature by linear regression. Then the 95% confidence interval of the estimated $\log(D_{temp})$ is estimated, and the 95% upper limit is used as a worst-case estimate for D at the temperature of heat treatment. This procedure allows worst-case estimation of D, and subsequently k, for specific pathogens at various temperatures, assuming temperature to be a main determinant of the inactivation rate.

Inactivation processes may theoretically result in less than 1 CFU·serving^{-1}. For example: if $N = 10^4$, a reduction of 10^6 results in 10^{-2} CFU·serving^{-1}. Practically, this is considered as 1 CFU per 100 servings, and the SIEFE model continues calculations with 1 CFU·serving^{-1} present after inactivation. The probability of organisms being present after inactivation is described by the parameter OC ('occurrence characterisation'). OC is defined as the logarithm of the theoretical amount of organisms present after inactivation (N_{ai}, 'ai' indicating after inactivation), as shown in equation 2.

$$OC = \log(N_{ai}) \qquad (2)$$

So, in the above example, $OC = -2$. If $N_{ai} \geq 1$, then $OC = 0$. If the reduction is 10^{20} or more, no organisms are assumed to be present anymore; reductions of 10^{20} or more are considered as complete inactivation. N_{ai} then is zero, and $OC = -\infty$.

3.4 Level 1: Hazard characterisation

A hazard entering a person by food consumption, may result in health-problems depending on the amount of the hazard consumed. Dose response data estimate the

Table 1. Attention values (*AV*), dose response parameters for infection (for the Exponential model and Beta-Poisson model), and mortality ratios for various bacteria. These dose response data result in an estimate of the probability of a certain health effect occurring, given consumption of a contaminated product

Bacteria	Source *	*AV* (CFU)	Exponential (r)	Beta-Poisson (α / β)	Mortality ratio [†,‡] (%)
Bacillus cereus	30	$1 \cdot 10^5$			0.0010
Campylobacter spp.	2, 33			0.039 / 55	0.28 [§]
C. jejuni	35	500			
C. jejuni A3249	39		$3.52 \cdot 10^{-6}$	0.145 / 7.589	
Clostridium botulinum type A	17, 38	100			15
C. perfringens	3, 35, 40	$1 \cdot 10^5$			0.10
Escherichia coli	3, 34	$1 \cdot 10^4$			0.30
E. coli O157:H7	40				2.0
L. monocytogenes	3, 8, 19	100	$1.179 \cdot 10^{-10}$		32
Plesiomonas shigelloides	39		$4.42 \cdot 10^{-10}$	0.057 / 1171	
Salmonella spp.	2, 33, 35	1		0.33 / 139.9	0.21[§]
S. meleagridis [‖]	39		$1.55 \cdot 10^{-6}$	0.428 / 8524	
S. typhi	33, 40			0.21 / 5531	6.0
S. typhi Quailes	39		$2.14 \cdot 10^{-8}$	0.203 / 29173	
Shigella spp.	33, 40			0.16 / 155	0.13
S. dysenteriae 1	33			0.5 / 100	
S. dysenteriae 1[¶]	39		$2.05 \cdot 10^{-4}$	0.157 / 9.16	
Staphylococcus aureus	3, 21	$1 \cdot 10^6$			0.10
Vibrio cholera classical	33			0.097 / 13020	
V. cholera El Tor	33			$2.7 \cdot 10^{-5}$ / 1.33	
V. cholerae 569b	39		$1.76 \cdot 10^{-9}$	0.508 / $7.52 \cdot 10^7$	
V. parahaemolyticus	2, 35	$1 \cdot 10^4$			0

* See References. 2 = Bean *et al.*; 3 = Bean and Griffin; 8 = Buchanan *et al.*; 17 = Hauschild; 19 = ICMSF; 21 = Jay; 30 = Notermans *et al.*; 33 = Rose and Gerba; 34 = Rose *et al.*; 35 = Shapton and Shapton; 38 = Ter Steeg and Cuppers; 39 = Teunis *et al.*; 40 = Todd.

† (Fatal cases)/(total cases of illness or intoxication for the organism)·100.

‡ If several values were given in literature, the worst case value was taken.

§ The average of deaths/cases for the organism of 1988-1992 was used.

‖ Pooled dataset of three strains.

¶ Pooled dataset for strains A-1 and M 131.

probability of infection and illness as a result of being exposed to a certain amount of pathogens. Level 1 uses first impressions of pathogens' infectivity, provided by attention values for infection and intoxication (*AV*). Attention values can be derived from e.g.: data on reported outbreaks, expert knowledge, microbiological norms, and *MID*-values (Minimal Infectious Dose). The attention values have been incorporated in the pathogen database. In case exposure to a hazard is close to, or greater than *AV* (CFU·serving^{-1}), the probability that problems will occur is realistic. In the literature, critical limits are sometimes given as CFU·g^{-1} or CFU·ml^{-1}, for example, Jay (21) reported for *Staphylococcus aureus* that: 'at least 500.000 to 1 million/g must be present in order to produce food poisoning symptoms in man.'. In level 1, risks are generally assessed for a serving size of 100 g, so for 10^6 CFU·g^{-1} *AV* should actually be 10^8 CFU·serving^{-1}. $AV = 10^6$ CFU·serving^{-1} is however used for *S. aureus* because of the safety perspective. Some examples of *AV* values for a variety of pathogens are shown in Table 1.

The *AV* is used to estimate the probability of foodborne illness as a result of consuming a certain concentration of a hazard. This probability is described by the *HC* value ('health problem characterisation'). *HC* is defined as the logarithm of the estimated concentration of the hazard in the product (*N*) divided by the *AV* for the organism (equation 3).

$$HC = \log\left(\frac{N}{AV}\right) \qquad (3)$$

If $N \geq AV$ then $HC = 0$. *HC* is a simple representation of the assumption of log-linearity of dose-response relations. For example, Buchanan *et al.* (8) also assumed log-linearity for the dose-response relation for *Listeria monocytogenes*.

3.5 Level 1: Risk characterisation

The SIEFE model uses characteristic numbers for risk characterisation. The formerly mentioned *OC* value describes the probability of occurrence of a hazard in the product, and the *HC* value characterises the probability of a negative response, given occurrence of the hazard.

Together they characterise the probability of foodborne illness as a result of consuming a certain product. A measure of this probability is the *PC* value ('probability characterisation'). *PC* is defined as the sum of *OC* and *HC* (equation 4).

$$PC = OC + HC \quad (4)$$

In level 1, probabilities are categorised ranging from very low to very high, using the *PC, OC,* and *HC* values. Since order of magnitude estimations are used in the first level of detail, characteristic numbers are used, for example: if $PC < -6$ the probability of having problems is considered to be very low; one in a million people will have problems as a result of consuming a serving (100 g) of the product (Table 2). The values were chosen by sensible reasoning, and can be changed if required.
After characterising the probability of foodborne illness by *PC*, risk-determining phenomena of production processes (RDP) are found by using the step characteristic *SC* (equation 5) and knowledge rules.

$$SC = \log\left(\frac{N_i}{N_{i-1}}\right) \quad (5)$$

SC estimates the logarithmic change in pathogens during a process step. Process steps that are characterised by a high value of *SC* are generally risk-determining. Growth and inactivation have been categorised by means of the absolute value of *SC,* ranging from low to complete growth and inactivation (Table 2).
The knowledge rules for selecting the RDP are explicitly mentioned in the procedure resulting in transparancy. Consequently, they can be criticised and changed if necessary. The knowledge rules are shown in Table 3.

Table 2. Categorisation of the probability of having problems, by characteristic numbers *PC* (probability characterisation), *OC* (occurrence characterisation), and *HC* (health-problem characterisation). Also shown is a characterisation of growth and inactivation, by *SC* (step characterisation). Absolute values of *SC* are given.

	PC, OC, and *HC*	SC_{growth}	$SC_{inactivation}$
very low	≤ -6		
low	$-6 < PC \leq -5$	< 0.3	< 1
moderate	$-5 < PC \leq -4$	$0.3 \leq SC < 1$	$1 \leq SC < 5$
high	$-4 < PC \leq -3$	$1 \leq SC < 5$	$5 \leq SC < 10$
very high	$-3 < PC \leq 0$	$5 \leq SC < 10$	$10 \leq SC < 20$
complete		≥ 10	≥ 20

Table 3. Knowledge rules to be used for support in establishing risk-determining phenomena and relevant scenarios in level 1 risk assessments according to the SIEFE model (for definitions: see Table 2)

1. If *PC* is very low, because of complete inactivation:
   1.1. study recontamination after inactivation.
   Since the inactivation is overwhelmingly large, it is not useful to study it more accurately. If recontamination occurs after inactivation, this may completely determine risk; if high growth occurs, or a highly infectious pathogen is concerned, the prevalence of recontamination is mainly important, if moderate or low growth occurs, the level of recontamination is important.
2. If *PC* is very low, because of high or very high inactivation:
   2.1. study recontamination after inactivation,
   2.2. study the parameters that determine growth,
   2.3. study the steps that result in the largest changes (see *SC* values).
   The hazard is inactivated to a very high extent. However, inactivation is not complete, and therefore it is interesting to study it more accurately. Recontamination in an almost sterile product may completely determine risk. Small changes in process parameters may well change growth opportunities after inactivation, and thereby result in risk-determining process steps, so it is interesting to study growth-determining parameters in a scenario.
3. If *PC* is very low, with moderate, or low, or no inactivation:
   3.1. study the initial contamination level,
   3.2. study the parameters that determine growth,
   3.3. study the steps that result in the largest changes (see *SC* values).
   The growth and inactivation kinetics appear not to be really relevant during the production process, so the initial contamination level can be risk-determining. Also, small changes in process parameters may well change growth opportunities, and thereby result in risk-determining process steps. For a scenario it is therefore interesting to study growth determining parameters.
4. If *PC* is moderate or low, because of high or very high inactivation, or complete inactivation with recontamination:
   4.1. study dose response data,
   4.2. study recontamination,
   4.3. study the steps that result in the largest changes (see *SC* values).
   The hazard is largely inactivated. However, remaining organisms or organisms that recontaminate the product after inactivation may be able to cause problems. Risk depends on which process step has the biggest influence under certain circumstances. Accurate dose response data may also be relevant, especially in the range of moderate risk estimates.
5. If *PC* is moderate or low, with moderate, or low, or no inactivation:
   5.1. study the initial contamination level,
   5.2. study dose response data,
   5.3. study the parameters that determine growth,
   5.4. study inactivation (if present),
   5.5. study the steps that result in the largest changes (see *SC* values).
   The initial contamination level may be risk-determining, since growth and inactivation may exclude each other's effects. More accurate dose response data may result in a different estimation of risk. Small changes in process parameters may well change growth opportunities, and thereby result in other risk-determining process steps (other steps showing the largest changes), so it is interesting to study growth determining parameters in a scenario. In level 1, inactivation is based on a worst-case value for the inactivation parameter k. *Risk estimates may be lower if specific values are used for* k.

Table 3 continued:

6. If *PC* is high or very high, because of high or very high growth:
   6.1. study (re)contamination,
   6.2. study inactivation (if present),
   6.3. study the steps resulting in the largest changes (see *SC* values).
   As organisms apparently are able to grow very well, prevalence of contamination, or re-contamination after inactivation may completely determine risk. In level 1, inactivation is based on a worst-case value of k. *Risk estimates may change if specific values are used, so it is interesting to re-estimate inactivation. Several inhibitory substances may result in less growth in practice, so the steps resulting in the largest changes are important to study.*
7. If *PC* is high or very high, with moderate, low, or no growth:
   7.1. study (re)contamination,
   7.2. study dose response data
   7.3. study inactivation (if present),
   7.4. study the steps resulting in the largest changes (see *SC* values).
   Prevalence of contamination, or recontamination after inactivation may completely determine risk, since the presence of the hazard, even in small amounts, results in high risk. Dose response data apparently are very important for risk. In level 1, inactivation is based on a worst-case estimate of k. *Risk estimates may change if specific values are used. It is therefore interesting to re-estimate inactivation.*

The first level's goal is to rank risks and efficiently find RDP, using values that are on the safe side. It is best to try several scenarios, and vary several parameters in the first level of detail, to be sure that no relevant RDP are overlooked. Van Gerwen *et al.* (44) show in the second part of their study the use of various scenarios in level 1, for two example products (chapter 6 of this thesis).

4. Level 2 risk assessment

The risk-determining phenomena, determined in level 1, are studied in a more quantitative way in the second level.

4.1 Level 2: Exposure assessment - process identification

In many cases, growth or inactivation are risk-determining aspects depending highly on temperature. For example in cooling processes, it may be important to study temperature-changes. Temperature-changes during cooling or heating have been included in the SIEFE model, and this chapter shows some practical examples of how to estimate temperature gradients.

Practical equations were derived for estimation of the temperature in the centre of (semi-)solid products, in batch systems without product convection, by Zwietering

and Hasting (49). For example, in case the external resistance is negligible (often the case in food heat treatments), the centre-temperature in a cylinder, with a height of two times the radius (R), can be described by:

$$T_{centre} = T_{ext} + (T_0 - T_{ext}) \cdot 2.0397 \cdot \exp(-8.2514 \cdot Fo)$$

T_0 is the initial temperature in the centre of the product, and T_{ext} is the temperature of the heating or cooling medium. This equation is valid for $Fo > 0.0864$, with Fo the Fourier number:

$$Fo = \frac{\lambda \cdot t}{c_p \cdot \rho \cdot L^2}$$

λ is the thermal conductivity of the product ($J \cdot s^{-1} \cdot m^{-1} \cdot K^{-1}$); t is time (s); c_p is the thermal coefficient of the product ($J \cdot kg^{-1} \cdot K^{-1}$); ρ is the product density ($kg \cdot m^{-3}$); and L is the characteristic dimension (m). In this example, L is equal to the radius. Values for λ, c_p, and ρ can be found in the literature for various products, see for example Tschubik and Maslow (42) and Mohsenin (28). If the values for a certain product are unknown, approximate values of similar products can be used. In many cases values for water, $\lambda = 0.6$ $J \cdot s^{-1} \cdot m^{-1} \cdot K^{-1}$; $c_p = 4200$ $J \cdot kg^{-1} \cdot K^{-1}$; $\rho = 1000$ $kg \cdot m^{-3}$ (1), can be used as a first approximation, since water is the major constituent of most food products. The Fourier number allows easy comparison of temperature changes in various shapes of food products. Growth and inactivation at changing temperatures are estimated by taking small time steps, assuming temperature to be constant per time step.

A second example is estimation of temperature-changes in a liquid product (convective heating). The internal heat transfer coefficient, α_i ($J \cdot s^{-1} \cdot m^{-2} \cdot K^{-1}$) of liquid products highly depends on the product and the process applied, for example: with, or without stirring; type of stirrer etc. α_i can be estimated with:

$$Nu = \frac{\alpha_i R}{\lambda}$$

Nu is the Nusselt number, and R and λ as described before. The Nusselt number indicates whether conduction or convection is predominant in the product. For example, in a batch system without stirring, it can be assumed that a product is convectively heated if $Nu > 2$, with (18):

Table 4. Example of contamination data stored in the food database

product/ingredient	genus	% positive samples*	number of samples*	concentration (CFU/g)	remark	ref.†
consumption milk	*Bacillus cereus*	35.7	157	$1 \cdot 10^4$		37
consumption milk	*Clostridium perfringens*			1	less than 1 spore per ml	12
consumption milk	*Clostridium tyrobutyricum*			0.2	less than 0.2 spore per ml	12
raw cow milk	*Bacillus cereus*	35	185			36
raw cow milk	*Bacillus cereus*	9	100	100		14
raw cow milk	*Clostridium botulinum*			0.001	less than 1 per l	12
pasteurised cow milk	*Bacillus cereus*	35		1000		37
pasteurised cow milk	*Bacillus cereus*	100	38	0.1		30
pasteurised cow milk	*Bacillus cereus*	35	100	1000		14
sterilised cow milk	*Bacillus cereus*	40	148			14
cheese	*Brucella abortus*	0	63			22
cheddar cheese	*Campylobacter jejuni*	0	127			22
cheddar cheese	*Clostridium botulinum*	0	40			12
cheddar cheese	*Bacillus cereus*	14	50	100		14
Gouda cheese	*Clostridium botulinum*	0	40			12
soft cheese	*Escherichia coli*	10	2000			22
soft cheese	*Listeria monocytogenes*	6	333	$1 \cdot 10^5$	from several countries	23
Oriental soft cheese	*Escherichia coli*	25	240			22

* In case a range of values was given, the worst-case value was included in the database.
† See References: 12 = Collins-Thompson and Wood; 14 = Doyle; 22 = Johnson *et al.*; 23 = Loncarevic *et al.*; 30 = Notermans *et al.*; 36 = Te Giffel *et al.*; 37 = Te Giffel *et al.*

$$Nu = 0.34(X)^{0.265} = 0.34\left(\frac{R^3 g\beta(T_{ext} - T_0)\rho^2}{\eta^2} \cdot \frac{\eta c_p}{\lambda}\right)^{0.265} \quad \text{if } 10^5 < X < 10^{10}.$$

g is the gravitational acceleration (9.81 $m \cdot s^{-2}$); β, the fluid volumetric expansion coefficient (K^{-1}); and η is the viscosity of the product ($Ns \cdot m^{-2}$). Values for η can be found in the literature for various products (42), or can be approximated. The value of β can be approximated by taking the value of water, for example $\beta = 2.06 \cdot 10^{-4}$ K^{-1} at 20^0C (1).

The relevance of α_i for total heat transfer in the product can be estimated by estimation of the total resistance (49). For this purpose, general values for the external heat transfer coefficient (α_e) for gas and liquids were derived from Beek and Muttzall (4), and have been stored in a database. If α_i is negligible in a non-stirred process, it certainly is negligible in a stirred process. Then, there is no reason describing it more accurately. Temperature changes in the product then depend on the heat transfer from heating medium to the product instead of heat transfer inside the product.

Growth and/or inactivation can be described in relation to these temperature changes.

4.2 Level 2: Exposure assessment - initial contamination

Contamination of food products can occur by contamination via raw materials (initial contamination) or recontamination during the production process. In practice, most raw materials are only analysed for several (groups of) microorganisms, for example, the total aerobic plate count, coliforms, and *Escherichia coli.* For specific hazards, contamination data are often unknown.

The SIEFE model uses data from various literature references to help the user in selecting realistic contamination levels and incidences for the specific product and hazard under study. These data are stored in a database. For example, data on contamination of pasteurised milk by *B. cereus* (Table 4) were reported by several authors (14,30,37).

If contamination level or incidence are risk-determining, and the estimates of contamination are uncertain, it is sensible to use a range of contamination data for calculation of exposure, for example N_0 = 1 to 10^4 CFU·serving^{-1}. Then, the importance of accurate estimation of initial contamination can easily be shown. Figure 3 shows

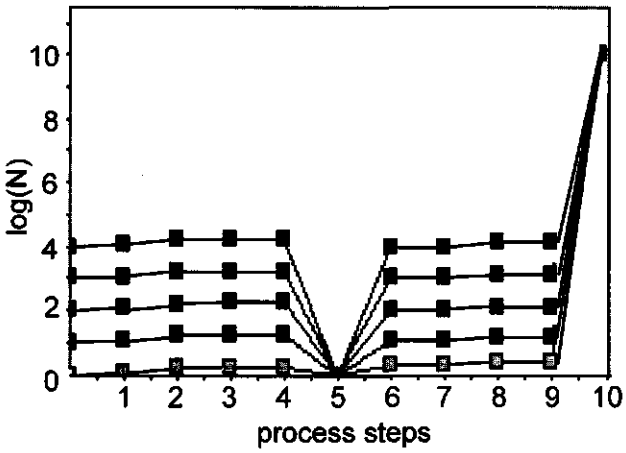

Fig. 3. The use of various scenarios for (re)contamination in exposure estimations. Initial contamination level varies between $\log(N_0) = 0$ (lower line) and $\log(N_0) = 4$ (upper line). During heat treatment (step 5), the pathogen is completely inactivated (reduction > 10^{20}), pictured as $\log(N) = 0$. Immediately after heat treatment, recontamination occurs. Recontamination level varies between $\log(N_{rec}) = 0$ (lower line) and $\log(N_{rec}) = 4$ (upper line). After recontamination, the maximum amount of pathogens ($N = 10^{10}$ CFU·serving^{-1}) is reached during storage (step 10).

an example where accurate estimation of initial contamination is not relevant, since the hazard is completely inactivated (reduction > 10^{20}) during heat treatment in step 5 for all scenarios of initial contamination from 1 to 10^4. This is an example where clearly it is detected that it is no use going more into detail estimating a value, even if a very inaccurate value is used.

4.3 Level 2: Exposure assessment - recontamination

Recontamination of the product occurs in many ways, for example by workers' hands, by microorganisms present in stagnant areas, and by contact surfaces. At present, quantitative models to estimate recontamination are scarce. Literature data and knowledge rules can however be used for support in estimating recontamination. De Wit and Kampelmacher (13) for example, reported amounts of pathogens present on hands of workers in various food industries. These data can be used, and have

been incorporated in the food database. The user can enter and update data, to extend and actualise the database. As for initial contamination, it is sensible to use a range of values for recontamination if the estimates are very uncertain. Figure 3 shows an example of the irrelevance of accurate estimation of recontamination. Whatever level of recontamination is used, in step 10 large growth takes place, and the product will be unsafe anyway. In this example, the probability of recontamination is the only relevant aspect, not the level of recontamination, nor the exact amount of organisms in the end product. This shows that by simulating several scenarios, it can be detected in every specific case, which is the variability of interest to concentrate on.

4.4 Level 2: Exposure assessment - inactivation and growth

In level 2, inactivation and growth can best be estimated using several models and comparing the results of the various models (46). Comparison of models is useful, as no model is able to accurately predict microbial responses under all circumstances, and it gives an indication of the accuracy and variability in estimation of growth and inactivation. Van Gerwen and Zwietering (46) showed several primary and secondary models that can be applied for general predictive purposes, and these models have been implemented in the SIEFE model. Also, literature references for specific growth and inactivation models (often response surface models) for various pathogens have been included in the SIEFE model.

Next to comparison of various models it is sensible to study the growth and inactivation parameters of the hazard more accurately. In level 1, worst-case estimates of these parameters were used, which may be too fail safe in the specific situation studied. The example of acid based spread presented in chapter 6 of this thesis confirms this.

4.5 Level 2: Hazard characterisation

In level 2 dose response curves are created based on available dose response parameters. In practice, there are relatively few dose response data to describe the probability of infection, for only a few infectious and toxico-infectious pathogens. Moreover, for those known, accuracy is often rather low, especially in the practically relevant low ranges. Problems related to the extrapolation of experimental results towards real-life situations have frequently been mentioned in the literature (6,8,15,20,26,33). Models to generate dose-response curves are for example the Exponential model and the Beta-Poisson model (15,39). Some reported dose response parameters for several bacteria are shown in Table 1. The dose response data result

in an estimate of P_h; the probability of a certain health effect, for example infection and illness, given consumption of a contaminated product.

Severity (S) is a phenomenon that has to be described quantitatively for estimation of the consumer's risk, regarding the CODEX (11) definition of risk: a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food (11). Severity can be considered as a weighing factor, enabling hazard and risk ranking, and facilitating health resource allocations. Murray (29) extensively described various approaches that have been developed to quantify the burden of disease, and Mauskopf and French (25) provide some severity data for *C. botulinum* and *Salmonella*. None of the formerly mentioned papers on quantitative risk assessment (7,9,30,32,41,43,47) included quantitative data on the burden of disease into the risk estimates. The CAST report (10) gives a rather straightforward, qualitative categorisation of severity, varying from 'mild, self-limiting, for ≤ 1 day' to 'severe, for months', that can be used as a practical tool for risk ranking.

Because of the formerly mentioned restrictions, it was decided to use $S = 1$ in the risk assessment. Actually, this results in an estimate of the probability of having problems, instead of *Risk*. Supplemented by the qualitative categories presented by the CAST report (10), the probability of having problems can still be used for risk and hazard ranking.

In level 2 use is made of available dose response parameters and foodborne outbreak data from the literature. The data are stored in the pathogen database. Again, new data can be entered, to extend and update the database.

4.6 Level 2: Risk characterisation

The risk characterisation procedure estimates the risk of a certain health effect occurring related to consumption of a certain food product. The consumer's risk of having problems after one consumption is:

$$Consumer\ Risk = P_e \cdot P_h \cdot S \qquad (6)$$

- P_e is the probability of a contaminated serving.
- S is a measure for severity. Since it is a weighing factor, it is dimensionless. Momentary, quantitative data are limited, and therefore it was assumed that $S = 1$.
- P_h is the probability of a certain health effect (h) occuring. P_h is based on dose-response data and data on foodborne disease outbreaks. For example, the probability of mortality can be described as (16,39):

$$P_h = P(i|e) \cdot P(ill|i) \cdot P(d|ill) \quad (7)$$

- $P(i|e)$ is the probability of infection, given a certain exposure (e). $P(i|e)$ can for example be estimated with the Beta-Poisson model.
- $P(ill|i)$ is the conditional probability of illness after infection. This probability was assumed to be independent of the ingested dose. If quantitative data are not available, $P(ill|i)$ is assumed to be one (worst-case).
- $P(d|ill)$ is the conditional probability of dying after developing disease. $P(d|ill)$ can for example be based on foodborne outbreaks of disease (Table 1). P_h is then estimated assuming that a constant fraction of the infected individuals suffers from severe outcomes.

Following equation 6, the risk of not having problems after n servings per year is:

$$Risk_{\text{no prob}} = (1 - P_e \cdot P_h \cdot S)^n \quad (8)$$

The risk of having one or more problems by consuming n servings of a certain product per year can therefore be estimated by:

$$Risk = 1-(1 - P_e \cdot P_h \cdot S)^n \quad (9)$$

Like in level 1 the quantitative determination of the mostly relevant phenomena is conducted.
If the estimates for P_h and P_e are very uncertain, it is best to estimate risk using various scenarios. This will be shown for cheese spread in chapter 6 of this thesis. By doing so, the importance of more accurate estimation of these parameters in the third level of detail can be tested. If parameter-estimates clearly affect risk estimations, it is sensible to study the parameters more accurately, for example by experimental studies, by more extensive literature search, by using very specific models, and/or by stochastic description of the parameters.

5. Conclusion

This chapter describes a method for microbial quantitative risk assessment for food products and production processes. The method has been implemented as a decision supporting expert system; the SIEFE model: Stepwise and Interactive Evaluation of Food safety by an Expert system.

The model is different from other approaches of microbial risk assessment in its use of various levels of detail to assess risks. This stepwise approach allows one to first focus on the main problems, and prevents getting caught in too much complexity. The first, rough level of detail results in a first estimate of risk and risk-determining phenomena (RDP). Process variations (failures) can easily be incorporated into this level, to make sure that no relevant RDP are overlooked. The first level of detail uses the easy characteristic numbers *SC, OC, HC,* and *PC* for illumination of the RDP and for risk ranking. The simplicity of the characteristic numbers makes them easy to understand, giving clear quantitative insight into production processes.

The second level of detail studies the RDP more accurately. Quantitative data from various references from the literature can be compared, as well as general and/or specific models for estimation of growth and inactivation. This allows studying the problem from various points of view, and estimation of risk on a broad basis. Uncertainty can be handled using several scenarios in risk-estimation. Then, the importance of variations in data and differences in model-estimates, and the necessity of application of frequency distributions in the third level of detail can be estimated. Variation of data allows consideration of effects of control measures and failures.

The model is interactive by the use of several knowledge sources. Expert and literature knowledge are captured in knowledge rules. Due to the clear definitions of the knowledge rules they can be criticised and changed if necessary. This supports the user in critically using the model, and thereby assessing realistic risks. The SIEFE model can best be used by experienced microbiologists, as they are able to make best use of the knowledge rules and can interpret the model's estimates with criticism. Combining the latter to the fact that the SIEFE model focuses on products and their production processes, the model can be valuable as some sort of member of a HACCP team. In this role it can be used proactively to support decisions on optimisation of production processes according to a certain risk.

The model's stepwise approach provides a way to retain a clear overview of the processes studied, by first selecting the quantitative most important phenomena with a structured method. Consequently, the SIEFE model does not necessarily focus on stochastic description of all variables. Only for the risk determining parameters stochastic assumptions have to be made, so that for these more effort can be used, since no time is wasted in looking for less relevant parameters and stochastic data. Besides the quantitative important phenomena, a very useful outcome of the SIEFE model is that quantitatively negligible aspects can be omitted based on explicit reasoning. This can help the process of risk assessments in the complex field of microbial food safety, containing much variability and uncertainty in many parameters. By

pinning down the problem, risk determining variability and uncertainty can be revealed and handled, resulting in realistic risk assessment.

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6

Application of the SIEFE model for stepwise microbiological quantitative risk assessment

Abstract

The effectiveness of a stepwise approach of microbiological quantitative risk assessment was shown for two products; an acid based spread and a cheese spread. Although the products seem to be similar in their production process and intended use, the risk-determining phenomena are determined to be different, resulting in different ways of handling microbiological hazards. For the acid based spread *Staphylococcus aureus* was studied as a hazard, and for the cheese spread *Clostridium botulinum* type A and proteolytic type B. After rough risk assessments, the detailed risk assessments showed that quantitative data for risk-determining factors are not always available. This did however not necessarily prevent risk estimation. Using ranges of values instead, even helped in gaining insight into the most relevant aspects. The stepwise approach is shown to efficiently highlight both risk-determining, and negligible factors. The examples showed that the SIEFE model provides necessary insight into production processes and risk-determining factors to both risk assessors and decision makers, and detects the most relevant lacunae.

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Table 1. Results of the hazard identification procedure (27) for an acid based spread, and results after application of knowledge rules

rough hazard identification	***detailed hazard identification***	***knowledge rules***			
		Type 1	***Type 2***	***Type 3***	***Type 1&2&3***
***	*Aeromonas* spp.				
	Alcaligenes spp.		X		
	Bacillus spp.	X	X		
	Bacillus anthracis	X			
	Bacillus cereus	X	X		
	Bacillus subtilis	X	X		
	Brucella spp.				
	Campylobacter spp.		X		
	Chromobacterium spp.		X		
	Clostridium spp.	X	X		
	Clostridium botulinum	X	X		
	Clostridium botulinum A	X	X		
	Clostridium perfringens	X	X		
	Corynebacterium spp.		X		
	Coxiella burnetii				
	Enterobacter spp.				
	Enterobacter sakazakii		X		
	Enterococcus spp.		X		
	Escherichia coli		X		
	Flavobacterium spp.		X		
	Klebsiella spp.				
	Klebsiella pneumoniae				
	Listeria monocytogenes		X	X	
	Mycobacterium spp.		X		
	Nocardia spp.		X		
	Pasteurella multocida		X		
	Plesiomonas shigelloides			X	
	Proteus spp.				
	Pseudomonas spp.		X		
	Pseudomonas aeruginosa				
	Salmonella spp.		X	X	
	Salmonella anatum		X		
	Serratia spp.		X		
	Shigella spp.		X	X	
	Staphylococcus spp.		X	X	
	Staphylococcus aureus	X†	X	X	X
	Streptococcus spp.		X		
	Vibrio cholerae		X		
	Yersinia enterocolitica		X		

*** No organisms were found in the database that were reported to have cause health problems related to acid based spread.

† *S. aureus* was not removed by type 1 rules, because of possible formation of heat stable enterotoxins.

1. Introduction

Quantitative risk assessment for food products is an emerging device for food safety management systems. Microbiological quantitative risk assessment has gained much interest in past years, and various examples of quantitative risk assessments have recently been published for various hazards and food products (2,5,17-19,25,26,32). For systematically assessing microbial risks for food products in general, Van Gerwen *et al.* (28) proposed a stepwise and interactive approach: the SIEFE model. SIEFE is an acronym for Stepwise and Interactive Evaluation of Food safety by an Expert system. This chapter describes the application of the SIEFE model for two examples: an acid based spread, and a cheese spread. The products appear to be similar in their production process and intended use, but the procedure determined that the microbiological hazards related to these products are different, and it was observed that different risk-determining phenomena are associated with the products. The purpose of this chapter is to demonstrate the usefulness of the stepwise approach to find risk-determining phenomena and estimate risks of production and consumption of food products.

2. SIEFE model example 1: acid based spread

2.1 Acid based spread: Hazard identification

In this example, bacterial risks are assessed for an acid based spread. As a start, the first two levels of detail of the hazard identification procedure presented by Van Gerwen *et al.* (27) were applied (Table 1). No obvious hazards were found, since no foodborne outbreak related to acid based spread was found in the database and has been reported to our knowledge. A more detailed selection was based on reported presence of pathogens on the ingredients of acid spread. The ingredients are: water, vegetable oil, white cabbage, gherkin, sugar, vinegar, milk powder, salt, red sweet pepper, onions, spices, lactic acid, starch, and thickeners. This selection identified 39 pathogenic bacteria as potentially hazardous. These pathogens can be introduced into the product, and may cause problems in the future. This list of potential problems was reduced to the relevant pathogens by using knowledge rules. *Staphylococcus aureus* was left after application of all types of knowledge rules of the hazard identification procedure: Type 1, rules concerning survival of pathogens; Type 2, general rules on pathogen characteristics; Type 3, rules concerning growth opportunities (27). Although *S. aureus* will not survive the pasteurisation process (Table 2), it may cause problems as a result of growth and heat stable toxin formation before inactivation. Consequently, it was not removed by Type 1 knowledge rules.

Table 2. Production process of acid based spread with *SC* (step characterisation) values for *Staphylococcus aureus*. Bold values indicate the most relevant steps. The value '0.00' means very little growth or inactivation that could not be expressed in two decimals

Process step	time	temp. (^{0}C)	*SC*	*SC* scenario as1 * †	*SC* scenario as2 ‡	*SC* scenario as3 §
mixing	10 min	20	0.01	0.01	0.01	0.01
homogenising	1 min	20	0.00	0.00	**1.72**	**4.31**
mixing	30 min	40	0.10	0.10	0.10	0.10
stock/magnet/deaerating	15 min	40	0.05	0.05	0.05	0.05
heating	1 min	92	**< -20**	**< -20**	**< -20**	**< -20**
packaging	3 min	88				
heating	10 min.	85				
cooling1	30 min	45		0.07		
cooling2	30 min	37		0.12		
storage	16 mnth	20		**10**		
N_{si}			0	1	0	0
N			0	10^{10}	0	0
AV			10^6	10^6	10^6	10^6
OC			-∞	0	-∞	-∞
HC ‖			-∞	0	-∞	-∞
PC before heat treatment ¶			-5.84	-5.84	-4.12	-1.53
PC			-∞	0	-∞	-∞
probability of having problems			low	very high	moderate	very high

* 1 CFU recontamination per serving at cooling1.
† *SC* = 10 was assumed to be a maximum value for *SC*. Moreover, it was assumed that the maximum concentration (CFU·serving^{-1}) is 10^{10}.
‡ Homogeniser not properly cleaned; product remains in homogeniser for 1 day.
§ Homogeniser not properly cleaned; product remains in homogeniser for 2.5 days.
‖ If $N/AV \geq 1$, then $HC = 0$ (28).
¶ This *PC* value is important to estimate because of potential heat stable toxin formation before heat treatment.

S. aureus was estimated to be able to grow in the end product (Type 3 knowledge rules), assuming a storage temperature of 20 ^{0}C, a pH of 4.25 (worst-case estimation: rejection-value of the product), and a water activity (a_w) of 0.95. The growth parameters of *S. aureus* were assumed to be: T_{min} = 7 ^{0}C, T_{opt} = 37 ^{0}C, T_{max} = 48 ^{0}C, pH_{min} = 2.6, pH_{opt} = 6.5, $a_{w,min}$ = 0.83 (11,13,16,22). The gamma model assumes symmetry for estimation of $\gamma(pH)$, so pH_{max} was calculated to be 10.4. The value for the optimum growth rate, μ_{opt} = 1.25 h^{-1}, was derived from the microorganism database of the Food Design Support System (FDSS), described by Wijtzes *et al.* (33).

2.2 Level 1: Exposure assessment - Acid based spread

The production process of acid based spread used in this example (Table 2) is based on a production process as applied in practice. Several assumptions were made: the temperature in the production environment is 20 ^{0}C; the pH of the bulk product before step 3 (= mixing, and addition of the vinegar and lactic acid) is 7.0 (worst-case estimation); one serving of acid based spread contains 20 g spread.

Growth and inactivation during the production process were estimated assuming first-order growth and inactivation kinetics. The inactivation parameter k was estimated first by linear regression on log(D) as a response variable of temperature. D values and related temperatures were taken from the literature (13). Second, the 95% confidence upper value of the estimated D at 92 ^{0}C ($D_{92}^{\#}$) was estimated, and third, the $k_{92}^{\#}$ value was calculated from $D_{92}^{\#}$, with $k_{92}^{\#} = \ln(10)/D_{92}^{\#}$ (28). This resulted in the worst-case estimate $k_{92}^{\#}$ = 46.47 min^{-1}. Process steps were characterised by SC (step characterisation). SC estimates the logarithmic change of pathogens during a process step; $SC = \log(N_k/N_{k-1})$ (28). SC values are shown in Table 2. It can be seen that *S. aureus* is completely inactivated during the heating step (SC < -20). Before heating, limited growth occurs ($\sum SC_{before\ heating} = 0.16$).

The probability of organisms being present after inactivation was described by OC (occurrence characterisation). OC is the logarithm of the theoretical amount of organisms present after inactivation (N_{ai}, '*ai*' indicating after inactivation); $OC = \log(N_{ai})$. In case of complete inactivation, $N_{ai} = 0$, so $OC = -\infty$ (Table 2), meaning a very low probability of occurrence (28). If recontamination occurs after inactivation (1 CFU·serving^{-1}), OC = 0, since $\log(N_{ai}) = 0$.

2.3 Level 1: Hazard characterisation - Acid based spread

Attention values (AV) were used for rough hazard characterisation. The AV gives a first impression on pathogens' infectivity, or the amount of pathogens related to dan-

gerous concentrations of toxin. *S. aureus* was assumed to have an *AV* of 10^6 CFU·serving^{-1} (14,28).

The probability of a negative response, given that the product is contaminated, was characterised by the *HC* ('health-problem characterisation') value. *HC* is the logarithm of the estimated concentration of the hazard in the product (*N*) divided by the attention value (*AV*) for the organism: $HC = \log(N/AV)$ (28). For the normal production process of acid based spread, $HC = -\infty$ (Table 2).

2.4 Level 1: Risk characterisation - Acid based spread

The probability of having problems is characterised by the *PC* ('probability characterisation') value; $PC = OC + HC$. *PC* for *S. aureus* was estimated to be $-\infty$ (Table 2), indicating very low risk (28). Moreover, on the basis of these calculations it is very unlikely that *S. aureus* forms a significant amount of heat stable toxin before heat treatment, since *PC* before inactivation was estimated to be -5.84. $PC = -5.84$ means a low probability of having problems, according to the categorisation of *PC* that was proposed by Van Gerwen *et al.* (28).

In the production process of acid based spread the heat treatment (Table 2) appeared to be a risk-determining process step. The reduction, that was estimated with a worst-case inactivation parameter, is overwhelmingly large, so it is not necessary to study it more accurately in level 2. The knowledge rules presented by Van Gerwen *et al.* (28) highlight for a very low risk, because of complete inactivation, that recontamination after complete inactivation may completely determine risk. Closer study is therefore sensible. The low risk, as a consequence of growth and toxin formation before heat treatment, is determined by the initial contamination level, and the parameters that inhibit growth and consequently toxin formation before the heat treatment.

Before studying the risk-determining phenomena more accurately in level 2, scenarios were applied in level 1, to get first impressions of the importance of varying parameters for risk. The theoretical scenarios were based on the knowledge rules presented by Van Gerwen *et al.* (28) and on sensible reasoning. The relevant results of the scenarios are shown in Table 2.

Scenario as1: recontamination (1 CFU·serving^{-1}) after heat treatment. This scenario results in a very high probability of having problems ($PC = 0$). *S. aureus* entering the product after heat treatment is well able to grow during 16 months of storage at room temperature; $SC = 10$, the maximum value of *SC*. Prevalence of recontamination after heat treatment and growth during storage are thus important risk-determining phenomena, that need further study. Recontamination after the first heat treatment (1

min, 92 ^{0}C), or during packaging is more likely to occur. This will however not result in problems, as *S. aureus* is completely inactivated during the subsequent heat treatment (10 min, 85 ^{0}C).

Scenario as2: homogenising; machine not thoroughly cleaned, so product remains for 1 day at 20 ^{0}C. This scenario results in a moderate probability of having problems ($PC_{before\ heat\ treatment}$ = -4.12). It is true that *S. aureus* is completely inactivated during heat treatment, but growth before heat treatment may lead to formation of heat stable toxin. According to the knowledge rules presented by Van Gerwen *et al.* (28), initial contamination level and dose response parameters are risk-determining in this situation. Also, the parameters that determine growth before inactivation are risk-determining.

Scenario as3: homogenising; machine not thoroughly cleaned, so product remains for a weekend (2.5 days) at 20 ^{0}C. This scenario results in a very high probability of having problems, $PC_{before\ heat\ treatment}$ = -1.53. If present, *S. aureus* is well able to multiply before heat treatment ($SC_{homogenising}$ = 4.31). It is therefore likely that a dangerous amount of enterotoxin is produced. If the contaminated product is hardly mixed during subsequent processing (before packaging), some jars of spread will contain an unacceptably high concentration of toxin. More accurate estimation of prevalence of contamination, and growth in the homogeniser are important in this scenario. It is clear that this type of simulations can help to set critical limits for processes.

As an example, level 2 exposure assessment will be performed for the formerly mentioned scenario as1.

2.5 Level 2: Exposure assessment - Scenario as1, Acid based spread

In level 2, we assumed that *S. aureus* is completely inactivated, and that recontamination occurs after heat treatment (Table 3, rows 2 and 3). Various phenomena can be omitted in this level, such as the heat treatment, the prevalence of initial contamination, and concentration. The risk determining phenomena are: prevalence of recontamination and growth during storage.

Since *S. aureus* intoxication only occurs if the organism is able to grow and form toxin, it is sensible to first study growth. This information is given by the SIEFE model as a knowledge rule. Another knowledge rule highlights the fact that growth may be inhibited or even prevented by the organic acids acetic acid and lactic acid (10,13,20). It is therefore sensible to first reconsider the growth parameter pH_{min}, before using and comparing various growth models. As explained by Van Gerwen *et*

al. (28), the SIEFE model's second level of detail studies growth by comparing various growth models.

In level 1, pH_{min} was assumed to be 2.6 (11). More accurate study of pH_{min} reveals that *S. aureus* was mentioned only to be able to grow at pH < 4.3, in presence of inorganic acids (13). Inorganic acids are not present in the spread, so most probably $pH_{min} > 4.3$. If $pH_{min} > 4.3$, the secondary gamma model (34), and the cardinal temperature and pH model (CTPM) (21) estimated growth rate to be zero for a product of pH = 4.25.

The USDA (U.S. Department of Agriculture) Pathogen Modeling Program, version 5.0 (PMP) could not predict growth at pH = 4.25, since the model limit is pH = 4.5.

The secondary models by Dengremont and Membré (8) and the model by Eifert *et al.* (10) could not be used since the pH was far below the model limits. This also holds for the predictions of lag time and growth rate by Sutherland *et al.* (23) and Walls *et al.* (31); there were no predictions at pH = 4.25.

It is remarkable that most models could not be used to predict growth of *S. aureus* at pH = 4.25. This may indicate that in former studies, media with pH < 4.5-5.0 were ignored; that growth was very difficult to measure; or that growth was very unlikely to occur. Considering the fact that the presence of acetic and lactic acid most probably results in extra hurdles for growth besides the low pH, growth of *S. aureus* was assumed not to occur in acid based spread (Table 3, row 5), in this level of detail.

Prevalence and level of recontamination were difficult to estimate, since no models or data were available. Since growth was estimated not to occur it is likely that recontamination is not very relevant for risk after all. A worst-case estimation of prevalence of recontamination, $P_e = 1$ (Table 3, row 4), was therefore used in the

Table 3. Relevant assumptions for estimation of risk for *Staphylococcus aureus* in acid based spread

Assumption	parameter	ill_{tox}	$mortality_{tox}$
1. The production process is shown in Table 2			
2. Complete inactivation during heat treatment			
3. Recontamination after inactivation			
4. Prevalence of recontamination; $P_e = 1$	P_e	1	1
5. No growth after recontamination because of low pH (4.25) and presence of the organic acids acetic acid and lactic acid			
6. No growth means no toxin formation, so the probability of intoxication; $P_h = 0$	P_h	0	0
7. $Risk = 1 - (1-P_e \cdot P_h \cdot S)^n$	*Risk*	0	0

first instance, and the recontamination level was estimated to be one CFU·serving^{-1}. If recontamination eventually turns out to be relevant, risk can be estimated using various scenarios to describe recontamination. Based on recontamination of one CFU per serving of 20 g, exposure resulting from consumption of a serving, e = 1 CFU.

2.6 Level 2: Hazard characterisation - Scenario as1, Acid based spread

No growth occurs after recontamination, and consequently it was assumed that no toxin is formed in the product. For that reason, the probability of intoxication was estimated to be zero: $P_h = 0$ (Table 3, row 6).

2.7 Level 2: Risk characterisation - Scenario as1, Acid based spread

Risk of having one or more problems by consuming n servings of a certain product per year can be estimated by (28): $Risk = 1-(1-P_e \cdot P_h \cdot S)^n$, with S the severity of the health effect. The risk of intoxication was estimated to be: $Risk_{tox} = 0$, since $P_h = 0$.

In this case, the level and prevalence of recontamination are not relevant for the problem, since the organism is not able to grow and form toxin. Even with higher contamination, no growth would not result in toxin formation.

The SIEFE model estimates risk, and provides a list of the assumptions the risk was based on. Insight in the assumptions for risk estimation is essential for interpreting risk. Table 3 summarises the relevant assumptions resulting in the estimated risk for intoxication. It is important to realise that the risk estimate is based on the knowledge that has presently been included in the SIEFE model's databases. The knowledge is not complete, and new insights into microbial behaviour may lead to different risk estimates in the future.

2.8 Level 3: Recommendations

The assumption of non-growth of *S. aureus* in the end product has great impact on the risk estimates. Growth was assumed not to occur because of the low pH (4.25) of the product. To be certain that growth can be omitted, it is sensible to perform challenge tests for *S. aureus* in the end product.

Microorganisms are known to be able to adapt to unfavourable conditions, for example in case of improper cleaning. *S. aureus* may adapt to the acid conditions of the spread, so there is no certainty that an organism that is not able to grow at present, will not be able to grow under the same conditions in the future. For example, *Clostridium botulinum* was generally accepted not to grow below pH 4.6, until Raatjes and Smelt (20) showed growth at pH 4.0. Shortly, *S. aureus* intoxication by

sandwich spread is well prevented and controlled today, but constant monitoring remains necessary to prevent problems for tomorrow.

The SIEFE model was applied to the production process of acid based spread. *S. aureus* was studied since it was the only hazard remaining after application of all types of knowledge rules in the hazard identification procedure (Table 1). In a next step, other hazards can be studied. For example, generally relevant hazards that are able to grow in the end product in case of recontamination; a combination of type 2 and 3 knowledge rules (Table 1). Various hazards have various risks and risk-determining phenomena, giving insight in how to control the process most efficiently.

Primary production and storage of ingredients were not included in the risk assessment. Since *S. aureus* toxin is heat stable, it is sensible to study toxin formation before heat treatment, during primary production and storage of ingredients. In this example, safety of the product was shown to be controlled well during the production process. Apart from studying other hazards, a next challenge can be safety control before the factory doors.

3. SIEFE model example 2: cheese spread

3.1 Cheese spread: Hazard identification

The second application of the SIEFE model will be performed for processed cheese (in this case cheese spread), defined by CODEX as 'a product made by grinding, mixing, melting, and emulsifying with the aid of heat and emulsifying agents, one or more varieties of cheese, with or without addition of milk components and/or foodstuffs'.

The hazard identification procedure, presented by van Gerwen *et al.* (27) was used to select relevant hazards for cheese spread. *Clostridium botulinum* type A and *C. botulinum* type B proteolytic were selected as the most obvious hazards, since these organisms were reported to have caused outbreaks related to the consumption of cheese spread in the past (3,15). It is sensible to first estimate risk for these pathogens, before focusing on other hazards.

3.2 Level 1: Exposure assessment - Cheese spread

The production process of cheese spread used in this example was based on the literature (4) and practice, and is shown in Table 4.

Growth and inactivation, and *SC* values were estimated. The growth characteristics of *C. botulinum* type A and type B proteolytic were assumed to be equal: T_{min} = 10 ^{0}C; T_{opt} = 35 ^{0}C; T_{max} = 50 ^{0}C; pH_{min} = 4.6; pH_{opt} = 7; $a_{w,min}$ = 0.93 (13,16,22).

Based on the assumption of symmetry, $\gamma(pH)$ was estimated with pH_{max} = 9.4. The optimal growth rate was derived from FDSS (33); μ_{opt} = 1 h^{-1}. The inactivation parameter $k_{145}^{\#}$ was estimated to be $1.82 \cdot 10^3$ min^{-1} for *C. botulinum* type A, and 671 min^{-1} for *C. botulinum* type B proteolytic. Inactivation data from ICMSF (13) were used for these estimations of *k*. *SC* values are shown in Table 4. *C. botulinum* type A is completely inactivated (*SC* < -20), and proteolytic type B is highly inactivated during heat treatment (*SC* = -9.61). The *OC* expresses the probability of occurrence after inactivation: $OC = \log(N_{ai})$ = -∞, and -9.35 respectively, meaning very low occurrence (28).

3.3 Level 1: Hazard characterisation - Cheese spread

For *C. botulinum*, *AV* was estimated to be 10^2 CFU·$serving^{-1}$ (24). The probability of a negative response, given that the product is contaminated, was estimated to be very high for *C. botulinum*: $HC = \log(N/AV) = \log(1.32/100) = -1.88$.

3.4 Level 1: Risk characterisation - Cheese spread

The probability of having problems, taking into account the probability of contamination, *PC* = *OC* + *HC*, was estimated to be very low for both organisms: *PC* = -∞ (-∞ - 1.88) for *C. botulinum* type A, and *PC* = -11.23 (-9.35 - 1.88) for proteolytic type B. Heat inactivation is obviously a risk-determining phenomenon that needs closer study for *C. botulinum* type B. For *C. botulinum* type A the reduction is overwhelmingly large, and closer study is not sensible. According to the knowledge rules presented by Van Gerwen *et al.* (28) other risk-determining phenomena are recontamination after inactivation, and growth-determining parameters after inactivation.

Since these calculations are based on just one set of conditions, several scenario's were used to determine whether the probability may change as a result of failures, and to determine critical steps in the production process. The scenarios were chosen on the basis of the risk-determining phenomena, and sensible reasoning. Table 4 shows the results of the scenarios for *C. botulinum* type B proteolytic.

Scenario cs1: heat treatment: 130 ^{0}C, for 3 seconds (4). This scenario results in a very high probability of having problems, *PC* = -2.91 for *C. botulinum* type A and -2.25 for proteolytic type B. The *AV* for *C. botulinum* is low, 10^2 CFU·$serving^{-1}$, so even with only one CFU·$serving^{-1}$ present, $HC = \log(1/10^2) = -2$. It is obvious that presence of the hazard, and *AV* are important for risk, and that a low *PC* can only be achieved by a low *OC* value. It is therefore sensible to study the prevalence of con-

Table 4. Production process of cheese spread with characteristic numbers. Bold values indicate the most relevant steps. The value '0.00' means very little growth or inactivation that could not be expressed in two decimals. The value '0' for *SC* values means no growth or inactivation

Process step	time	temp. (°C)	*SC* type A/B	*SC* type B scenario cs1*	*SC* type B scenario cs2†	*SC* type B scenario cs3‡	*SC* type B scenario cs4§
trimming cheeses	1 h	20	0.07	0.07	0.07	0.07	0.07
blending	2 h	20	0.15	0.15	0.15	0.15	0.15
shredding (grinding, milling)	15 min	20	0.02	0.02	0.02	0.02	0.02
addition emulsifiers	15 min	20	0.02	0.02	0.02	0.02	0.02
heat treatment	2 s	145	**< -20/-9.61**	**-0.63**	**< -20**	**-9.61**	**-9.61**
creaming	30 min	90	0.00	0.00		0.00	0.00
packaging	10 min	70	0.00	0.00		0.00	0.00
cooling1 ‖	15 min	37	0.10	0.10		0.10	0.10
cooling2	15 min	20	0.02	0.02		0.02	0.02
storage	8 weeks	10	0	0		**10**	**10**
N_{ai}			$0/10^{-9.35}$	0.427	0	$10^{-9.35}$	1
N			1.32	1.32	0	10^{10}	10^{10}
AV			10^2	10^2	10^2	10^2	10^2
OC			-∞/-9.35	-0.37	-∞	-9.35	0
HC			-1.88	-1.88	-∞	0	0
PC			-∞/-11.23	-2.25	-∞	-9.35	0
probability of having problems			very low	high	very low	very low	very high

* Heat treatment at 130 °C, for 3 seconds.
† Heat treatment at 145 °C, for 6 seconds.
‡ Non cooled storage at 20 °C.
§ Recontamination after heat treatment (1 CFU·serving^{-1}), non-cooled storage at 20 °C.
‖ Stepwise cooling was assumed.

tamination, dose response data, and the extent of inactivation more accurately for this scenario.

Scenario cs2: heat treatment: 145 ^{0}C, for 6 seconds. This scenario results in a very low probability of having problems ($PC = -\infty$). *C. botulinum* is completely inactivated ($SC < -20$), so heat treatment is risk-determining. The inactivation is however so overwhelmingly large that it needs no further study. For this scenario it is interesting to study recontamination more accurately, since this will probably completely determine risk.

Scenario cs3: storage 20 ^{0}C (compared to 10 ^{0}C), for 8 weeks. This scenario results in very low probabilities for *C. botulinum* type B, $PC = -9.35$. If $N \geq AV$, as in this scenario, then $HC = 0$ (28), so $PC = OC + HC = OC$. Consequently, inactivation is an important risk-determining phenomenon in this scenario. If organisms remain in the product after inactivation (theoretically one in $10^{9.35}$ products), or recontamination occurs, the organisms are well able to grow at this temperature ($SC = 10$, the maximum value of *SC*). It is therefore sensible to also study growth during storage and (re)contamination more accurately (28). Other phenomena can be ommitted.

Scenario cs4: heat treatment: 145 ^{0}C, for 2 seconds, recontamination after inactivation, and non-cooled storage at 20 ^{0}C. As expected, this scenario results in very high probabilities for the hazards ($PC = 0$). Recontamination was assumed to occur, so $OC = 0$ in this scenario (28). Heat treatment is therefore not relevant for risk. Prevention of recontamination clearly is important, especially in case of non-cooled storage ($SC_{storage} = 10$). Storage and prevalence of recontamination are risk-determining phenomena in this scenario, that need to be studied more accurately. NB. This scenario has the same results if complete inactivation occurs.

Heat treatment appeared to be risk-determining in various scenarios, given that recontamination does not occur afterwards. If recontamination does occur however, it is very important for risk, especially if non-cooled storage (≥ 10 ^{0}C (16)) applies. As an example, level 2 risk assessment will be performed for scenario cs3, for *C. botulinum* type B proteolytic; this is the most heat resistant of the hazards under study.

3.5 Level 2: Exposure assessment - Scenario cs3, Cheese spread

Assuming scenario cs3, heat treatment, the prevalence of (re)contamination, and growth during storage are the risk-determining phenomena that need further study in this level.

Inactivation of *C. botulinum* type B proteolytic was studied by using the exponential model as the primary model. Tailing was not taken into account, because there was no indication of deviations from log-linearity during heat inactivation at 145 ^{0}C.

As in level 1, the worst-case value for $k_{145}^{\#} = 671$ min^{-1} was used, and the log reduction was estimated to be 9.61. In level 2, inactivation was estimated using both $k_{145}^{\#}$, and the point estimate k_{145}. The point estimate k_{145} was calculated as $k_{145} = \ln(10)/D_{145}$, where D_{145} was estimated from the linear regression line of D values given by ICMSF (13). $D_{145} = 1.05 \cdot 10^{-3}$ min, so $k_{145} = 2.19 \cdot 10^{3}$ min^{-1}, and the log reduction >> 20. Considering the fact that both log reductions of 9.61 (by the worst-case value), and of >> 20 are very high, it was decided to consider the inactivation as being complete (Table 5, row 2). Because of this complete inactivation, it is no use studying prevalence of contamination more accurately.

Growth during storage was estimated assuming the product characteristics: temperature = 20 ^{0}C, pH = 6.0, water activity (a_w) = 0.975. The following primary models were compared: the lag-exponential model (29), the Baranyi model (1), and the reparameterized Gompertz model (35). The secondary growth models that were compared are: the gamma model (34), and the CTPM (21). The comparisons showed that there were no relevant differences between these models; they all estimated growth to be very high ($N = N_{max} = 10^{10}$).

The Central Composite Model (TCCM) and The Extended Total Model (TETM) of Ter Steeg and Cuppers (24) were developed to estimate the time to a 100-fold increase (t_{100}) of proteolytic *C. botulinum* in cheese spread. The models predicted $t_{100} = 1.7$ and $t_{100} = 3$ weeks respectively, so also too large growth in 8 weeks of storage.

The USDA Pathogen Modeling Program version 5.0 (PMP) could not be used, since $a_w = 0.975$ was outside the model limits. The Tanaka model as described by Ter Steeg and Cuppers (24) could not be used either, since this model was only applicable for 30 ^{0}C.

Based on the above results we concluded that it is likely that much growth occurs during 8 weeks of storage at 20 ^{0}C. Using the simplest models available: the exponential and gamma models, growth was estimated to be $N/N_0 = 10^{10}$. The actual value of N is not relevant, since growth is too high anyway. The exposure was therefore estimated as: $e = 10^{10}$ CFU·serving^{-1} (Table 5, row 5).

The user is informed that it is important to realise that the presence of various specific growth inhibiting substances is not included in most growth models, and consequently growth estimates are fail safe.

The prevalence of recontamination is an important risk-determining factor in this example. If recontamination occurs after complete inactivation, *C. botulinum* type B proteolytic is well able to grow in the cheese spread used in this example. Recontamination is most likely to occur during the creaming step. During creaming, the cheese is in an open vessel, and pre-cooked cheese, or 'rework' is added to achieve the proper texture (4). The texture is normally checked by placing a knife into the vessel, which of course may be an important cause of recontamination. The prevalence of recontamination of cheese spread was however difficult to estimate, since no specific quantitative data on this subject were found in the literature. The only data found on prevalence of *C. botulinum* in cheese spread were that 'none of 10 samples of cheese spread were found to contain spores of *C. botulinum*' (7), and that 'Millions of jars of vacuum-packed cheese spread have been consumed and in only one jar was *Clostridium botulinum* toxin detected.' (30). Additionally, a knowledge

Table 5. Relevant assumptions for estimation of risk for *Clostridium botulinum* type B proteolytic in cheese spread

Assumption	parameter	ill	mortality
1. The production process is shown in Table 4			
2. Complete inactivation during heat treatment			
3. Recontamination after inactivation			
4. Prevalence of recontamination ranges from $P_e = 10^{-20}$ to $P_e = 1$	P_e	10^{-20} - 1	10^{-20} - 1
5. Very high growth after recontamination, resulting in an estimated exposure; $e = 10^{10}$ CFU per serving	e	10^{10}	10^{10}
6. The organism is very well able to grow, and therefore also able to form toxin			
7. Since $e = 10^{10} >> AV = 10^2$, it was assumed that every serving contains significant amounts of toxin, so the probability of intoxication; $P(i\|e)_{tox} = 1$	$P(i\|e)_{tox}$	1	1
8. Illness will definitely occur after consumption; it was assumed that $P(ill\|i) = 1$	$P(ill\|i)$	1	1
9. The value for the mortality ratio, given illness; $P(d\|ill) = 0.15$	$P(d\|ill)$		0.15
10. Severity was estimated as $S = 1$	S	1	1
11. Amount of servings consumed per year in the Netherlands varies between: $n = 10^6$ and $n = 10^9$	n	10^6 to 10^9	10^6 to 10^9
12. $Risk = 1 - (1 - P_e \cdot P(i\|e) \cdot P(ill\|i) \cdot P(d\|ill) \cdot S)^n$	*Risk*	Figure 1	Figure 1

rule was presented mentioning that most European type B strains are non-proteolytic (9), which is relevant for European markets. It was expected that prevalence is low, but quantitative estimation of prevalence (P_e) was not possible with the available information. For that reason, risk was estimated with P_e ranging from 10^{-20} to 1 (Table 5).

3.6 Level 2: Hazard characterisation - Scenario cs3, Cheese spread

The probability of infection, illness, or mortality can be estimated by dose-response parameters for the hazard. In this example however, no dose response data for *C. botulinum* type B proteolytic were available besides the *AV* of 10^2 CFU·serving^{-1}. Since the estimated amount of CFU·serving^{-1} ($e = 10^{10}$) is much higher than the *AV*, it was assumed that all servings contain toxin if they are contaminated, and P($i|e$) for intoxication was estimated to be 1 (Table 5, row 7).

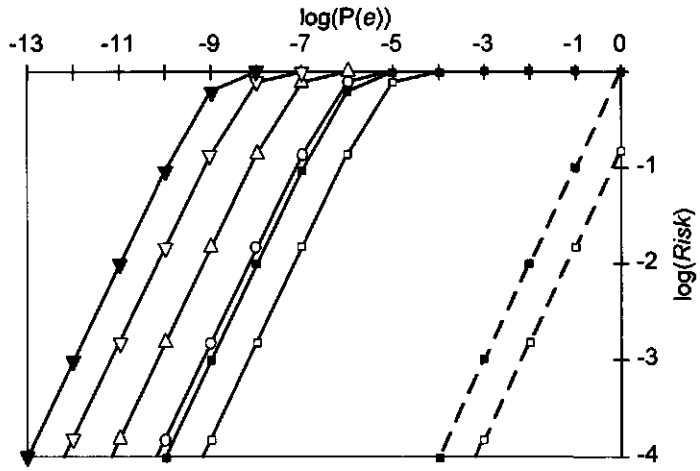

Figure 1. Log(*Risk*) of having one or more problems per year as a function of the logarithm of the prevalence of recontamination, log(P_e), for *Clostridium botulinum* type B proteolytic in cheese spread. Log(*Risk*) for death (mortality ratio P($d|ill$) = 0.15) is represented by □ for n = 10^6; ○ for n = 10^7; Δ for n = 10^8; ∇ for n = 10^9. log(*Risk*) for illness is shown by ■ for n = 10^6; and ▼ for n = 10^9. The dashed lines represent log(*Consumer Risk*) for illness (■), and for death (□), for consumption of one serving of cheese spread.

Intoxication was assumed to definitely result in illness, so P(*ill*|*i*) = 1. If ill, the probability of dying was estimated as the mortality ratio; P(*d*|*ill*) = 0.15 (12).
Severity (*S*) was assumed to be one. The qualitative categories presented in the CAST report (6) indicated severe problems, for week to months.

3.7 Level 2: Risk characterisation - Scenario cs3, Cheese spread

The cheese used in this example is a hypothetical cheese, since its characteristics were based on literature. Consequently, it was not known how many jars of the cheese spread are sold per year in the Netherlands. For that reason, *n* was assumed to vary between 10^6 and 10^9 servings per year.
Risk as a function of P_e and *n* is shown in Figure 1, and relevant assumptions for estimation of risk are shown in Table 5. For example, if prevalence is 10^{-9} (1 in 10^9 jars contains 1 CFU), and 10^8 servings are consumed per year, the probability that one or more people die in a year in the Netherlands as a result of consuming cheese spread is $1.49 \cdot 10^{-2}$ (Figure 1). This probability can be interpreted as one or more people dying of *C. botulinum* intoxication by this product every 70 years.
The *Consumer Risk* of dying from eating one serving of cheese spread is $P_e \cdot P(i|e) \cdot P(ill|i) \cdot P(d|ill) \cdot S = P_e \cdot 1 \cdot 1 \cdot 0.15 \cdot 1$. This shows that risk is totally determined by prevalence, and that really low frequencies (unmeasurable) should be achieved to control safety by frequency. Concerning the good safety record of process cheese products (7,30), P_e is most probably very low.

3.8 Level 3: Recommendations

Risk was estimated with the assumption that *C. botulinum* type B proteolytic is well able to grow in the product. Since growth estimates are probably worst-case, it is sensible to check growth experimentally by challenge testing in level 3.
The quantitative estimation of risk appeared to rely to a high extent on the prevalence of recontamination, P_e, and the amount of servings consumed, *n*. Risk estimates should therefore be based on more accurate estimation of these parameters. It normally is rather easy to estimate *n*, since sales data are generally known for most products per time period. Variations in *n*, due to for example fluctuating sales numbers, can be included in the risk assessment by describing *n* as a stochastic parameter. Given the accuracy of prevalence estimates this is probably not really relevant.
P_e and variations in P_e are more difficult to estimate, since no specific quantitative data on P_e are available, and very low frequencies are practically impossible to measure. Regarding food safety control it is more sensible to focus on a composition that does not allow growth of *C. botulinum* in cheese spread or measures that prevent

recontamination, than focusing on accurate quantitative estimation of the prevalence of recontamination.

4. Conclusion

The chapter showed stepwise quantitative microbial risk assessment for the products acid based spread and cheese spread. The first, rough level of detail provided risk-determining phenomena for the products and first estimates of risk. It was shown that the risk-determining phenomena related to the normal process can be used to find relevant failure scenarios. Concerning the efficient search for risk-determining phenomena and failure scenarios, the first level of detail can be a useful tool in finding Critical Control Points, as part of the HACCP system. The opportunity for easy hazard and risk ranking in the first level of detail was not shown in this chapter. If risks were assessed for various hazards, they could have been ranked according to food safety relevance by the *PC* (probability characterisation) values. The insight into the separate process steps, by means of *SC* (step characterisation) values, together with the *PC* values, can support management decisions on control strategies.

The risk-determining phenomena were studied more accurately in the second level of detail. It was for example shown that growth and inactivation can be studied by comparing various models. The second level of detail showed that quantitative estimates of risk-determining phenomena are not always available, because of lack of specific quantitative data or lack of models and model parameters. It was shown that this lack does not necessarily prevent risk estimation. Worst-case estimates were shown to be sufficient for some risk-determining phenomena, whereas other risk-determining phenomena were described by a range of values. If relevant, the worst-case, or ranging estimates can be studied more accurately in the third level of detail: by stochastic description, by experiments, or by renewed literature search.

The stepwise approach provides insight into risk and risk-determining phenomena, without unrealistic pretences of accurate estimation of the actual risk. If the risk-determining phenomena cannot be estimated quantitatively with the available data or models, their importance for risk can be shown by application of a wide range of values. If no quantitative data or models are available to estimate risk-determining phenomena, it is no use pretending accurate quantitative estimation by using frequency distributions. Moreover, it is useless to search for proper frequency distributions and related parameters for various aspects, if these aspects turned out to be hardly relevant for risk using simple calculations. The stepwise approach provides

this necessary quantitative insight into production processes to both risk assessors and decision makers.

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7

Comparison of two approaches for microbiological quantitative risk assessment

Abstract

Quantitative risk assessment for foodborne microbiological hazards has been recognised as an important research area. Various approaches for microbial quantitative risk assessment for food products have been developed in the past years. This chapter compares two of these approaches; the approach of Whiting and Buchanan, 1997, Int. J. Food Microbiol. 36:111-125, is compared to the SIEFE model presented by Van Gerwen *et al.* submitted 1999, chapter 5 of this thesis. Whiting and Buchanan's example of *Salmonella enteritidis* in mayonnaise was studied by the SIEFE model, and following the results of both approaches were compared and differences interpreted. The study clearly showed the necessity of definition of assumptions in risk assessments, since these clearly affect estimates throughout the risk assessment. Also shown was the importance of input data for risk estimates, and thereby the relevance of input data being well described and traceable. Inclusion of a *Salmonella* survival model by Whiting and Buchanan showed to affect exposure estimates; SIEFE does not contain a survival model. The most evident difference is the fact that SIEFE does not use stochastic variables in risk assessment in the first instance, whereas Whiting and Buchanan do. To our opinion SIEFE provides more quantitative insight into the production process of mayonnaise than Whiting and Buchanan, by highlighting aspects that are quantitatively important. These aspects can be focused on, and irrelevant parameters can be omitted.

1. Introduction

Microbial quantitative risk assessment (mQRA) is an emerging tool that can be applied in food safety management. General features and benefits of mQRA have frequently been described (20,21,27,29), as well as approaches for the use of mQRA for food products in general (23,42). and specific applications (2,4,5,22,28, 30,32,39,40,46). The stepwise approach of Van Gerwen *et al.* (42), is different by not necessarily focusing on stochastic description of all variables, but by first selecting the quantitatively most important phenomena with a structured method. This involves transparancy in risk assessment, and allows omission of less relevant aspects. Only for the relevant parameters stochastic assumptions have to be made. More effort can be used to focus on these parameters, since no time is wasted in looking into less relevant parameters and stochastic data.

Based on the CODEX definition of quantitative risk assessment (6), the stepwise approach (SIEFE model) consists of the steps hazard identification, exposure assessment, hazard characterisation, and risk characterisation (Fig. 1). SIEFE is an acronym for Stepwise and Interactive Evaluation of Food safety by an Expert system (42). First, rough risk assessment is performed (level 1) to find risk-determining phenomena. These phenomena can then be studied more accurately in level 2, for example by comparison of various growth and inactivation models, and the use of plausible ranges for parameters as a sort of sensitivity analysis.

The SIEFE model focuses on products and their production processes. The main goal of the SIEFE model is obtaining clear quantitative insight into production processes for support in decision making.

In this chapter the possibilities of the SIEFE model are illustrated by means of an example. In addition, the model is compared with one of the specific applications of mQRA described in the literature; mQRA for the product mayonnaise, made of pasteurised liquid eggs, has been described by Whiting and Buchanan (46) before. Following, the work of Whiting and Buchanan is indicated as W&B.

2. Hazard identification

The hazard identification procedure presented by Van Gerwen *et al.* (41) selects bacterial hazards for food products in general. For mayonnaise, obvious hazards following from this procedure were *Salmonella enteritidis*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, and *Proteus vulgaris*. These organisms have been reported to have caused foodborne outbreaks related to mayonnaise in the past (9,24,

31,38). It is sensible to start risk assessments for these pathogens, before focusing on other hazards. In this study, we focused on *S. enteritidis*, as W&B did in their paper.

3. Level 1: exposure assessment

The various steps of exposure assessment are: process identification; (re)contamination; and growth and inactivation (Fig. 1). The characteristic numbers presented in the following sections (*SC, OC, HC, PC*) were introduced and explained by Van Gerwen *et al.* (42).

3.1 Level 1 exposure assessment: process identification & (re)contamination

The production process of mayonnaise was assumed to be similar to the steps used by W&B. Two steps were added: packaging of the liquid egg bulk into containers after heat treatment, and mixing of the ingredients for preparation of the mayonnaise (Table 1). In their paper, W&B studied effects of variations in the temperature of heat treatment (59 ^{0}C instead of 60 ^{0}C) and the temperature of storage (11 ^{0}C instead of 6 ^{0}C). Likewise we studied these variations (Table 1). Several assumptions for the process were made: (i) no recontamination after pasteurisation; (ii) the volume of an egg is 40 ml; (iii) contamination before pasteurisation occurs anyway, with initial contamination, N_0 = 1 CFU·serving^{-1}; (iv) a serving of mayonnaise contains 10 ml of

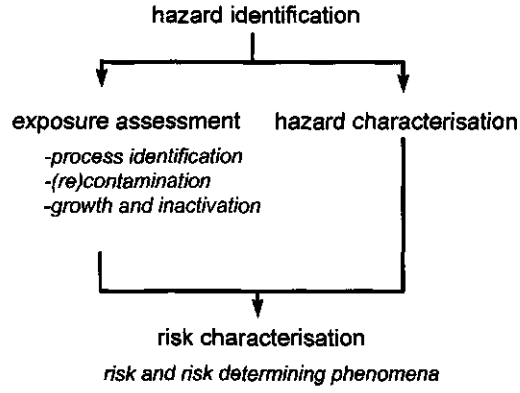

Fig. 1. Framework of the procedure for quantitative risk assessment.

Table 1. Process of pasteurisation and storage of eggs, supplemented by making, and storage of mayonnaise. Also shown are *SC* values * for *Salmonella enteritidis*, for the normal process; for a process with mild heat treatment (heat); and for a process with mild heat treatment and mildly cooled storage (abuse). The value '0.00' means very little growth or inactivation that could not be expressed in two decimals. Bold values highlight important process steps.

Process step	time	temp. (^{0}C)	pH	*SC* *	temp. (^{0}C)	SC_{heat}	temp. (^{0}C)	SC_{abuse}
heat treatment	3.5 min	60	7.0	**-1.31**	59	-0.83	59	-0.83
packaging	5 min	20	7.0	0.01				
storage1	6 days	6	7.0	0.36			11	**4.45**
storage2	4 hours	20	7.0	0.65				
mixing	15 min.	20	3.9	0.00				
storage3	1 day	20	3.9	0.22				
N_{ai} † (CFU·serving^{-1})				0.049		0.15		0.15
N (CFU·serving^{-1})				17.38		17.38		$2.14 \cdot 10^5$
AV				25		25		25
OC				-1.31		-0.83		-0.83
HC				-0.16		-0.16		0 ‡
PC				-1.47		-0.99		-0.83
probability of having problems				very high		very high		very high

* *SC* is a characteristic number for process steps: $SC = \log(N_k/N_{k-1})$

† 'ai' means after inactivation. Estimate based on initial contamination level, N_0 = 1 CFU·serving^{-1}.

‡ If $N \geq AV$, then $HC = 0$.

pasteurised liquid egg; (v) the pH of the product is 7.0 until mixing, afterwards pH = 3.9, due to the addition of vinegar in the preparation of mayonnaise; (vi) the water activity of the product is 0.997 throughout the process. W&B's scenario of high initial contamination was not included. If this is relevant, the initial contamination level is highlighted as a risk-determining factor by the knowledge rules presented by Van Gerwen *et al.* (42).

3.2 Level 1 exposure assessment: growth and inactivation

Growth and inactivation were estimated assuming first order kinetics. The growth rate μ (min^{-1}) was calculated with the gamma model (48). The growth characteristics of *S. enteritidis* were assumed to be equal to those of *Salmonella* spp.: T_{min} = 4 ^{0}C; T_{opt} = 37 ^{0}C; T_{max} = 47 ^{0}C; pH_{min} = 3.8; pH_{opt} = 7.25; $a_{w,min}$ = 0.92; μ_{opt} = 1.65 h^{-1} (8,19,25,37,47). pH_{max} was estimated as $2 \cdot pH_{opt} - pH_{min}$, by assuming symmetry for calculation of $\gamma(pH)$. For estimation of the inactivation parameter k (min^{-1}), (i) D values were taken from the literature (3,11,12,15-17,19,26,36); (ii) D was described as a response variable of temperature by linear regression; (iii) the 95% confidence interval was estimated at the temperature (T) of heat treatment; and (iv) the 95% upper limit was used as a worst-case estimate of k ($k_T^{\#}$) , This resulted in the worst-case estimate $k_{60}^{\#} = 0.86$ min^{-1}.

SC (step characterisation) is a characteristic number describing changes in microbial load during process steps. From the *SC* values (Table 1), it was clear that no relevant growth or inactivation occurred during the normal production process; $|SC| = 1.31$ at maximum, indicating twentyfold change only. It can be observed that the *SC* value transforms time and temperatures into one value that can be used to compare the effects of various process steps; in this example, 6 days at 6 ^{0}C and 4 h at 20 ^{0}C gave the same order of magnitude for growth. Table 1 shows that heat treatment at 59 ^{0}C did not really affect the outcome of the process, but that storage1 at 11 ^{0}C resulted in a high SC_{abuse} value (4.45).

The *OC* expresses the probability of presence after inactivation ('ai'); it is the logarithm of the theoretical amount of organisms present after inactivation, $\log(N_{ai})$. For example, $OC = \log(Nai) = -2$ means that one in 100 servings contains one CFU. In the example of *S. enteritidis* in mayonnaise, $OC = \log(0.049) = -1.31$ (Table 1). This means a very high probability that *S. enteritidis* will be present after the heat treatment of the normal process according to the interpretation of the SIEFE model's characteristic numbers, presented by Van Gerwen *et al.* (42),.

Table 2. Summary of the fraction of positive eggs reported in literature, the number of eggs tested, and the concentrations of *S. enteritidis* found in the eggs.

Reported fraction of contaminated eggs	Number of eggs tested	Amount (CFU/egg)	Ref. *
0.009	3,000		10
0.002	1,000		10
0.002	1,000		10
0.001	1,000		10
0.003	3,000		10
0.002	1,000		10
0.0003			10
0.0009	3,210		10
0.0005			10
0.001			18
0.006	5,790		18
0.004	3,659	20 †	18
0.005	1,603	100,000 ‡	18
0.009	1,952		18
0			46
0.19			46
0.00114	880		7
0.000758	1,320		7
0.000368	2,720		7
0.000329	3,040		7
0.006	8,698		13
		20 †	13
0.0006			34
0.004	250		45
0.00806	372		33
0.00701	998		33
0.011	451		14
0.103	68		14
0.011	667		14
0.009	452		14
		10 §	14

* See references: (10) Henzler *et al.*; (18) Humphrey *et al.*; (46) Whiting and Buchanan; (7) de Louvois; (13) Humphrey; (34) Poppe; (45) Vugia *et al.*; (33) Perales and Audicana; (14) Humphrey *et al.*.

† This amount was measured within 3 weeks after laying.

‡ This is a worst-case value for eggs stored for 3 weeks or longer.

§ Measured in 100 ml.

4. Level 1: hazard characterisation

A first impression of *S. enteritidis* infectivity was provided by the attention value (*AV*). *AV* was assumed to be 25 CFU·serving^{-1} (worst-case), based on outbreak data for ice cream (44).

The probability of having health-problems, given that the product is contaminated, was estimated to be very high for the normal process: $HC = \log(N/AV) = \log(17.38/25) = -0.16$, meaning that the level of the hazard per serving was around the attention value.

5. Level 1: risk characterisation

The probability of having problems, taking into account the probability of contamination, was $PC = OC + HC = -1.47$, meaning a very high probability. According to the knowledge rules presented by Van Gerwen *et al.* (42), and given the characteristic numbers calculated here, the prevalence of contamination, and dose response data should be studied more accurately, as well as the inactivation step. Table 1 shows that rapid growth took place at mildly cooled conditions (11 ^{0}C) during storage1, so the abuse scenario was also included in level 2. The estimated very high probability of having problems resulted from a rather rough and worst-case risk assessment. The estimate is fail-safe, and should be considered as an indication that high risk may arise from consuming a serving of mayonnaise. A closer study of risk-determining phenomena in level 2 of the SIEFE model was therefore sensible.

6. Level 2: exposure assessment

Following from the level 1 analysis of the mayonnaise, level 2 exposure assessment consisted of a more accurate study of the risk-determining phenomena: prevalence of contamination; inactivation; and growth during storage1 at different temperatures.

6.1 Level 2 exposure assessment: contamination

Contamination data on *S. enteritidis* in eggs from the literature are shown in Table 2. The fraction of contaminated eggs ranges from 0 to 0.19 with the majority of data ≤ 0.011. The big differences in the data, and the relevance of the fraction of contaminated eggs for risk urged us to use a plausible range for this fraction in risk assessment. The fraction of contaminated eggs ($P_{e,cont}$) was assumed to vary between 10^{-4} and 1 (Tables 3 and 4, line 1).

6.2 Level 2 exposure assessment: inactivation

Based on the assumption of first-order kinetics, the exponential model (43) was used as the primary model. Tailing was not taken into account, as cell populations of less than 10^7 CFU·ml^{-1} *S. enteritidis* were mentioned to result in virtually linear survivor curves (11).

The average estimation k_{60} was calculated as $k_{60} = \ln(10)/D_{60}$, with D_{60} being estimated from the linear regression line of D values from the literature (3,11,12,15-17,19,26,36). D_{60} = 0.49 min, so k_{60} = 4.7 min^{-1}, and the log reduction = 7.1. In comparison, Blackburn *et al.* (3) showed predictions of D_{60} values in whole egg of 0.41 (k_{60} = 5.6 min^{-1}) and 0.43 min (k_{60} = 5.4 min^{-1}), resulting in 8.51 and 8.21 log reductions respectively.

Considering the large difference between k_{60} = 4.7 min^{-1} (average) and $k_{60}^{\#}$ = 0.86 min^{-1} (worst-case, level 1), it was decided to use both values for risk assessment. Also, k_{59} was estimated, since W&B concluded inactivation temperature (59 ^{0}C or 60 ^{0}C) to be important for risk; k_{59} = 3.0 min^{-1}. This resulted in a 4.5 log reduction. The use of both the average (k_{60}), and the worst-case ($k_{60}^{\#}$) estimates provided insight into the relevance of k for risk, being some sort of sensitivity analysis.

It was assumed that the eggs were processed within 3 weeks after laying, and that the contamination level was less than 20 CFU/egg (13,18). One serving of mayonnaise then contained 5 CFU = 0.70 log CFU (Tables 3 and 4, line 2). The 7.1 (average), 1.3 (worst-case, level 1), and 4.5 (average at 59 ^{0}C) log reductions resulted in less than one CFU per serving of mayonnaise; log(CFU·serving^{-1}) = -6.40, -0.611, and -3.78 respectively (Tables 3 and 4, line 3).

If a serving theoretically contains less than one CFU, the probability of a serving containing one CFU ($P_{e,inact}$) can be estimated. In this example log $P_{e,inact}$ = -6.40, log $P_{e,inact}^{\#}$ = -0.611, and log $P_{e,inact,59}$ = -3.78 (Tables 3 and 4, line 4).

6.3 Level 2 exposure assessment: probability of exposure

In the SIEFE model, the sum of log $P_{e,cont}$ and log $P_{e,inact}$ results in the log probability of being exposed to a certain hazard, log P_e (Tables 3 and 4, line 5). The large variation in the fraction of contaminated eggs urged us to use a range of values for P_e anyway. Very accurate estimation of the extent of inactivation was therefore not necessary at this stage.

6.4 Level 2 exposure assessment: growth

After heat treatment, N < 1 CFU·serving^{-1} for every scenario (so $P_{e,inact}$ < 1, see 6.2), and the SIEFE model continued calculations for 1 CFU·serving^{-1} (42). Growth was

estimated using the exponential and gamma models, for the normal process; storage1 at 6 ^{0}C, and for the abuse process, in which storage1 is at 11 ^{0}C. As in level 1, this resulted in log CFU·serving^{-1} = 1.24 for the normal process (Table 3, line 8), and log CFU·serving^{-1} = 5.33 for the abuse process (Table 4, line 8). Since we used simple models these are rough estimates. The qualitative conclusions on growth are that the normal process results in low growth, and the abuse process results in high growth.

7. Level 2: hazard characterisation

For dose-response assessment, the infectivity of *S. enteritidis* was assumed to be typical of other salmonellae. The following parameters were used: r = 0.00752, for the exponential model; α = 0.33 and β = 139.9 for the beta-poisson model (35). The dose-response models resulted in an estimation of the probability of infection (i), given exposure (e). Ingestion of log(N) = log(e) = 1.24 (normal process) resulted in log P($i|e$) = -0.91 for the exponential, and -1.42 for the beta-poisson model, so about 10% probability. Ingestion of log(N) = 5.33 (abuse process) resulted in log P($i|e$) = 0, and -0.04 respectively, so about 100% probability. Since the differences between the models were rather small, we used the exponential model in further calculations (Tables 3 and 4, line 9), as W&B did.

It was assumed that infection definitely results in illness; P($ill|i$) = 1 (worst-case), so the probability of illness, P_{ill} = P($i|e$)·P($ill|i$) = P($i|e$) (42). The death rate was estimated as the average of death rates of reported cases for the period 1988-1992 for *Salmonella* spp. (1), P($d|ill$) = 0.0021. The probability of death can then be estimated as: P_{mort} = P($i|e$)·P($ill|i$)·P($d|ill$) = P($i|e$)·0.0021 (not included in Tables 3 and 4).

8. Level 2: risk characterisation

Risk was estimated for consumption of one serving of mayonnaise: *consumer risk* = $P_h \cdot P_e \cdot S$. P_h is the probability of a certain health effect (h) occurring, such as illness or death. S is a measure for severity, and is assumed to be one (42). *Consumer risk* as a function of P_e is shown in Fig. 2. Obviously, log(*consumer risk*) was highly affected by P_e. More precisely, the inactivation parameter k showed to highly affect log *consumer risk*. Fig. 2 also shows that the difference between the mildly cooled, and the normal process is negligible compared to the differences by inactivation, and the difference between illness and mortality.

Table 3. Comparison of the results of the quantitative risk assessment of the SIEFE model and of Whiting and Buchanan (46) (W&B) for *Salmonella enteritidis* in mayonnaise for the **normal** production process as shown in Table 1.

	S2n *	#S2n †	WBn ‡
1. Log probability of positive serving - log $P_{e,cont}$	-4 .. 0	-4 .. 0	
2. Initial *S. enteritidis* in pooled eggs §	0.70	0.70	-1.6
3. Post pasteurisation §	-6.40	-0.611	-9.7
4. Log probability of positive serving (SIEFE) or container (W&B) after pasteurisation - log $P_{e,inact}$	-6.40	-0.611	-6.7
5. Log probability of *S. enteritidis* positive serving - log P_e	-10.4 .. -6.4	-4.6 ..-0.61	-8.7 ‖
6. Post storage §	0.38		-3
7. Post home storage §	1.02		-0.8
8. Mayonnaise at consumption §	1.24		-3.6
9. Log probability of positive serving being infectious- log P(*i*|*e*)	-0.91		-4.8
10.Log probability of illness from entire process - log *consumer risk*	-11.3 .. -7.3	-5.5 .. -1.5	-11.5

* S2n = SIEFE model level 2, normal process.
† # means the worst-case situation, where k_{60} # = 0.86 min^{-1}.
‡ WBn = Whiting and Buchanan (46), normal process.
§ log(*N*), W&B: log CFU·g^{-1}; SIEFE: log CFU·$serving^{-1}$
‖ Own calculation. The level of contamination post pasteurisation = -9.7 log CFU·g^{-1} = -8.7 log CFU·$serving^{-1}$. Log P_e then is -8.7.

Table 4. Comparison of the results of the quantitative risk assessment of the SIEFE model and of Whiting and Buchanan (46) (W&B) for *Salmonella enteritidis* in mayonnaise for the **abuse** process as described in Table 1.

	S2a *	S2ai †	WBa ‡
1. Log probability of positive serving - log $P_{e,cont}$	-4 .. 0	-4 .. 0	
2. Initial *S. enteritidis* in pooled eggs §	0.70	0.70	-0.39 ‖
3. Post pasteurisation §	-6.40	-3.78	-4.7
4. Log probability of positive serving (SIEFE) or container (W&B) after pasteurisation - log $P_{e,inact}$	-6.40	-3.78	-1.7
5. Log probability of *S. enteritidis* positive serving - log P_e	-10.4 .. -6.4	-7.8 .. -3.8	-3.7 ¶
6. Post storage §	4.46	4.46	2.3
7. Post home storage §	5.11	5.11	3.6
8. Mayonnaise at consumption §	5.33	5.33	0.68
9. Log probability of positive serving being infectious- log P(*i*|*e*)	0	0	-0.53
10.Log probability of illness from entire process - log *consumer risk*	-10.4 .. -6.4	-7.8 .. -3.8	-2.3

* S2a = SIEFE model level 2, abuse process (storage1 at 11 ^{0}C). The differences between S2a and the worst-case situation, where k_{60} # = 0.86 min^{-1}, were comparable to the normal process (Table 3), and therefore the worst-case situation is not shown.

† i means inactivation at 59 ^{0}C, instead of 60 ^{0}C.

‡ WBa = Whiting and Buchanan (46), scenario 4 (abuse)

§ log(*N*), W&B: log CFU·g^{-1}; SIEFE: log CFU·serving^{-1}

‖ One of the scenarios applied by W&B concerned a high level of infected flocks and abuse of eggs. This scenario was not considered in this chapter, since level 1 calculations start at 1 CFU·serving^{-1} anyway. If relevant, the contamination level is highlighted as risk-determining by the knowledge rules presented in chapter 5 of this thesis.

¶ Own calculation. The level of contamination post pasteurisation = -4.7 log CFU·g^{-1} = -3.7 log CFU·serving^{-1}. Log P_e then is -3.7.

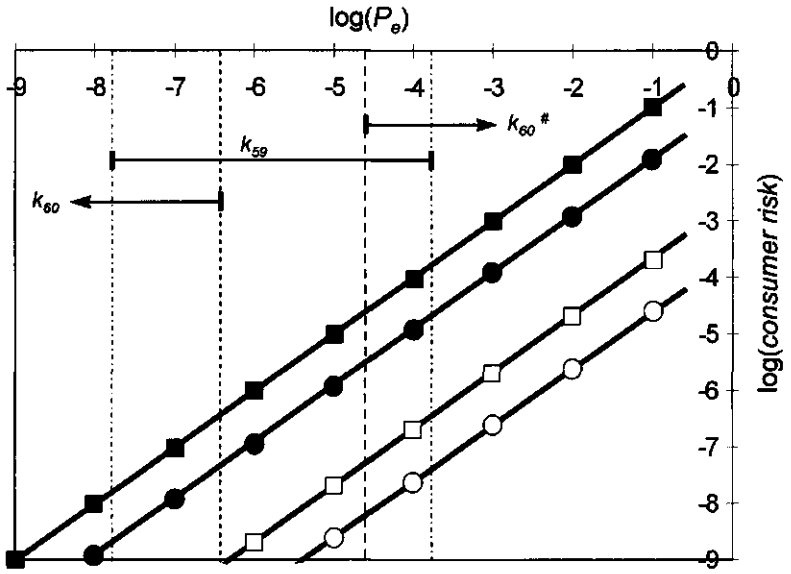

Fig. 2. Log(*consumer risk*) as a function of the logarithm of the prevalence of contamination, log(P_e), for *Salmonella enteritidis* in mayonnaise. ■ represents the situation with mildly cooled storage1 (abuse). The ranges of P_e and *consumer risk* are also shown in Table 4, lines 5 and 10. ● represents the normal situation, and ranges of P_e and *consumer risk* are shown in Table 3, lines 5 and 10. □ and ○ show log(*consumer risk*) for death (mortality ratio P(*d*|*ill*) = 0.0021).
The arrows (⟵| and |⟶) and the horizontal line (|⟷|) indicate the range of log(P_e) related to the average k (k_{60}), the worst-case k ($k_{60}^{\#}$), and the average k at 59 °C (k_{59}) respectively.

9. Comparison of two approaches for microbial quantitative risk assessment

Tables 3 and 4 show results of both models. W&B's results are median values. Comparing SIEFE level 2 normal process (S2n) to W&B normal process (WBn) shows close risk estimates; S2n: -11.3 .. -7.3, WBn: -11.5 (Table 3, line 10). Despite similarity in risk estimates, there is a substantial difference in log P(*i*|*e*); -0.91, and -4.8 respectively (Table 3, line 9). This large difference can partly be explained by the fact that W&B assume 10^{-3} CFU·g^{-1} in a container after heat treatment, whereas

we assumed 1 CFU·serving^{-1}. If W&B's results are presented per serving, post storage (Table 3, line 6) = 0, instead of -3; post home storage = 0.8 + 3 = 2.2; and mayonnaise at consumption = -3.6 + 3 = -0.6 log CFU·serving^{-1}. The probability of a serving being infectious then is log P($i|e$) = log(1-exp(-0.00752·$10^{-0.6}$) = -2.8. Since the log probability of a positive serving (log P_e) is -8.7 (Table 3, line 5), the resulting risk = -8.7 - 2.8 = -11.5 (exactly the same).

Moreover, W&B included a *Salmonella* survival model to predict the development of the organism during storage of the mayonnaise. The survival model affects the estimated concentration of pathogens that is consumed. If expressed as log CFU·serving^{-1} (see above), the differences in post home storage (S2n: 1.02, WBn: 2.2) are smaller than the differences in mayonnaise at consumption (1.24 vs. -0.6).

SIEFE level 2, normal, worst-case (#S2n) compared to WBn shows a considerable distinction in risk estimates, -5.5 .. -1.5 and -11.5 respectively (Table 3, line 10). This distinction is obviously due to the difference in estimated log reduction during heat treatment; #S2n: 0.70 - (-0.611) = 1.31, and WBn: -1.6 - (-9.7) = 8.1. Also, there is a substantial difference in log P($i|e$); explanation see above.

SIEFE level 2, abuse (S2a) and WBa show large differences in risk estimates: -10.4 .. -6.4 and -2.3 respectively (Table 4, line 10). The inactivation estimates, S2a = -7.1 and WBa = -4.3, are one reason, the initial contamination level is another reason for the different risk estimates. W&B's estimate of initial contamination combined both initial contamination level (CFU·g^{-1}), and prevalence of contamination, resulting in -0.39 log CFU·g^{-1} = 0.61 log CFU·serving^{-1} (Table 4, line 2). If prevalence is also included for the SIEFE model (Table 4, line 1) the initial contamination level would be -4 .. 0 + 0.7 = -3.3 .. 0.70. This shows clearly that the estimate used in W&B's approach is in the lower range. In addition, it is remarkable that W&B base their risk estimates on the log probability of a positive container after pasteurisation, $P_{e,inact}$ = -1.7, instead of the log probability of a positive serving, log P_e (-3.7). This is one difference in the approach of how to estimate risk.

The distinction in risk estimates between SIEFE level 2, abuse process, with inactivation at 59 ^{0}C (S2ai) and WBa (Table 4, line 10) are mainly due to the above mentioned initial contamination level and the approach of how to estimate risk.

In their conclusion, W&B highlighted the relevance of the differences in heating, and storage1 temperature for risk estimates. The SIEFE model confirmed the relevance of the heating temperature. The temperature during storage1 appeared to be negligible compared to the relevance of the inactivation parameter k. For SIEFE, the 10^6 fold difference between the worst-case and average inactivation estimates at 60 ^{0}C

overwhelmed all other aspects. The worst-case estimate was based on the 95% upper confidence limit of the estimated D at 60 ^{0}C. This means a 0.025 probability of k values resulting in this kind of worst-case estimates. These k values result in log *consumer risk* for illness is ≥ -4.6, and for death ≥ -7.3 at abuse conditions (Fig. 2). Consequently, there may be a serious problem especially at high prevalence of initial contamination. For more accurate risk estimations, it is sensible to verify the present inactivation data. This can be done by literature study on heat resistance of the specific strains that are actually present in the eggs, or by specific experiments in the product. Stochastic description of both prevalence of contamination, and the inactivation parameter k is sensible to determine the consequences of additive effects on risk.

10. Conclusion

Most approaches of microbial quantitative risk assessment (mQRA) that have been published so far start with stochastic description of parameters for risk estimation. The SIEFE model is different, since it does not start with stochastic assumptions for parameters, instead it may demonstrate that in some cases stochastic description of various parameters is not necessary. The simple start relates to the main benefit of this approach; obtaining clear quantitative insight into the process of risk assessment.

Comparison of the two approaches for mQRA clearly demonstrated the relevance of the steps in risk assessment. Both SIEFE and W&B estimate the probability of illness of consuming a serving of mayonnaise, containing 10 ml of liquid pasteurised egg. W&B based the estimate on the probability of presence of the hazard in a container (1000 g), and on the level of the hazard per gram ($CFU \cdot g^{-1}$). In contrast, SIEFE estimated every step per serving of consumed end product. These assumptions not necessarily result in different risk estimates, but definitely affect the intermediate estimates. Definition of assumptions in this field is a must, and short evaluation of the consequences of the assumptions is desirable.

The importance of input data for risk estimates was also shown. The inactivation estimates stress this most obviously; our data set of D values from the literature resulted in a much wider confidence interval at 60 ^{0}C than W&B's data, with important consequences for the risk. This highlights the fact that risk estimates can only be interpreted well if input data and sources are well described and traceable.

W&B included a *Salmonella* survival model into risk assessments, which affected the estimated concentration of pathogens consumed. Exclusion of survival models

result in worst-case estimates. This is all right for rough risk estimates, but more accurate risk assessment would definitely benefit from inclusion of survival models.
An evident difference between both approaches is the fact that SIEFE used deterministic variables for risk estimation, whereas W&B used stochastic variables. To our opinion, SIEFE provided clear quantitative insight into the process of risk assessment. In this case it was clearly shown that the variances in k, and the additive effects of prevalence of contamination and inactivation are relevant. Inherently, SIEFE highlighted those aspects for which stochastic description is relevant. A third level of detail of SIEFE can then perform the necessary simulations. An additional advantage of this approach is that focus on irrelevant parameters is prevented.
Comparison of various approaches is useful in the complex field of mQRA; it can strengthen qualitative conclusions in case of similar results, or provide new insight into other risk related phenomena in case of different results. In this study, the differences between two approaches of mQRA demonstrated that assessed risks highly depend on the data used, and the assumptions made. This is a clear evidence of the necessity of transparency in QRA.

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8

Discussion: The SIEFE model as a tool in the production of safe food

1. Introduction

Quantitative risk analysis is a phenomenon that cannot be set aside in today's world. Numerous disciplines, varying from nuclear science to banking, use principles of quantitative risk analysis, to weigh policies, and to make decisions. For some disciplines quantitative risk analysis has been an integrated part for years, such as the space industry, whereas in other disciplines it is an emerging phenomenon. For example, quantitative risk analysis related to the food product's safety actually is in its infancy in the daily practice of food production.

Quantitative risk analysis consists of quantitative risk assessment, risk communication, and risk management. The work in this thesis concerns the first part; quantitative risk assessment (QRA), and focuses on food products. Food safety may be jeopardised by microbial, chemical, or physical hazards. Microbial QRA (mQRA) has been of current interest, and various approaches for mQRA have been developed, and described in the literature recently (e.g. 1,2,8,12,17). The method for mQRA presented in this thesis, the SIEFE model, distinguishes itself by assessing microbial risks with a stepwise approach; first rough risk assessment before going into detail. This stepwise approach provides clear quantitative insight into microbial contamination, growth and inactivation during production processes, and prevents getting caught in too much details.

Up till now the mQRA approach for food products strictly focuses on microorganisms, and the possible use of the proposed approach to chemical and physical hazards was not appraised. This chapter tentatively evaluates the applicability of the SIEFE model for chemical and physical hazards. Similarities and differences between microbial, physical and chemical hazards are described, and the impact on the rough level of detail of the SIEFE model is evaluated.

The chapter ends by reviewing the features of the SIEFE model for food production practice, and describes future opportunities and needs for (m)QRA.

2. SIEFE model, physical and chemical hazards

2.1 Physical and chemical hazard identification

The SIEFE model's hazard identification procedure for bacteria is described in chapter 2 of this thesis. In this paragraph, it is considered whether this procedure is also applicable to physical and chemical hazards. The SIEFE model considers hazard identification as the identification of agents capable of causing adverse health effects and which may be present in a particular food. It is important to realise that this is a product-oriented approach. In contrast, many chemical hazard identifications are health-effect oriented (14,21).

Physical hazards are particles or foreign material in finished products (6,13) that may cause adverse health effects. Chemical hazards consist of food additives (e.g. colours, preservatives), contaminants (e.g. environmental contaminants, food packaging migrants), residues (e.g. pesticides, veterinary medicines), natural compounds (e.g. plant toxins, antinutritional factors), adulterants, and malicious tampering (14) that adversely affect human health.

For identification of physical hazards there are many clinical reports on case histories (4,11). As for microbial hazard identification, this allows selection of the most obvious hazards for a food product. A more detailed hazard identification consists of hazards that are likely to be introduced into the product, and accordingly may cause problems in the future. For microbial hazards, this detailed hazard identification selects pathogens that are present in the ingredients of the product. A similar approach can be used for physical hazards. For example, Lewis (7) lists sources of physical hazards in food production environments that can serve as a guide for the detailed hazard identification.

For chemical hazards, the SIEFE model's approach of selecting the most obvious hazards by case histories is difficult to apply in practice, since cases where food chemicals have been implicated as acute causes of human illness are very rare (14). For example, for chronic effects, such as cancer, it may be very difficult to link the disease to consumption of a specific food product. Those chemical hazards that have proven links between disease and consumption of specific products of course should be selected as obvious. For example peanut residues causing severe health effects in people with peanut allergy. Other obvious hazards can be the hazards of public concern, or generally well-known hazards related to the product. Examples of these types of hazards are food additives in general, anti-nutritional factors in plant components, and pesticide residues in fruits and vegetables. A more detailed hazard identification could be based on food surveillance data of known chemical hazards. For example, in the Netherlands, monitoring data of agricultural products are re-

viewed annually by a collaborative programme of the Ministry of Agriculture and various other agricultural bodies (KAP report) (18).

2.2 Physical and chemical exposure assessment

As for microbial hazards, it is sensible first to roughly study exposure, to distinguish between relevant and negligible aspects. In chemical risk assessment this is known as a tiered approach to prioritise food chemicals for detailed assessment (3).

2.2.1 contamination

For microbial hazards the SIEFE model's rough exposure assessment assumes that the product is contaminated anyway, which can also be assumed for physical and chemical hazards. The SIEFE model starts with the minimum contamination size of 1 CFU·serving^{-1}, so the focus is on the change in concentration of microorganisms during a production process. A similar approach can be followed for chemical and physical hazards. Nevertheless, for physical and chemical hazards it is difficult to use practically relevant minimal values for contamination level, since physical hazards are described by length (m), and chemical hazards by concentration (g·serving^{-1}). For example, a microbiological hazard (discrete) of 0.5 CFU does not exist in practice, but a physical hazard (continuous) of 0.5 mm in length is well possible.

Instead of minimal levels, for various chemical hazards maximum levels can be applied as starting levels. The fact is that various chemical hazards have known maximum allowed concentrations, for example the Maximum Residue Level (MRL), in mg·kg^{-1} food. Changes in concentration can be modelled from these levels. Hazards that are formed in, or migrate into the product during processing start at a level of 0 mg·serving^{-1}.

It should be realised that the first level of detail's focus is on finding risk-determining phenomena. Accurate estimation of the starting levels is therefore not necessary in this stage.

2.2.2 'growth and inactivation' - kinetics

For physical hazards instead of growth and inactivation, increase and decrease in size are relevant. Increase in size of a physical hazard is generally unlikely. Once present, the hazard may stay intact or be ground, and can be removed by filtering, magnets, or automatic visual recognition and consequent removal. For risk assessment, changes in size during the production process can be described. A characteristic value similar to the formerly mentioned *SC* (step characterisation) can be used to describe the changes in size throughout the process. It is likely that there will be par-

ticles that slip through the process of removal or grinding. The failing ratio of removal or grinding can be expressed by a characteristic number, such as the formerly mentioned *OC* (occurrence characterisation). For microbiological hazards, the *OC* is expressed as the logarithm of the value. As shown later, it is not logical to use a logarithmic expression for physical hazards.

A chemical hazard's change in concentration during a production process can be quantitatively described using simple models, and can be expressed by *SC*. As for microorganisms, orders of magnitude of heat inactivation or production can be estimated by assuming first-order kinetics. The dependence of the inactivation rate k can be described by the Arrhenius equation: $k = k_{\infty} e^{\frac{-E_a}{RT}}$, with k_{∞}, the inactivation rate at infinite temperature (s^{-1}); E_a the activation energy ($J \cdot mol^{-1}$); R the gas constant: 8.314 $J \cdot mol^{-1} \cdot K^{-1}$; and T, temperature (K). Migration of chemicals into the product can be described by Fick's law: $\phi_m = -ID\frac{\partial c}{\partial x}$, with ϕ_m, the diffusion-rate ($mg \cdot m^{-2} \cdot s^{-1}$); ID, the diffusion-coefficient ($m^2 \cdot s^{-1}$); and $\partial c/\partial x$, the driving force ($mg \cdot m^{-4}$). For order of magnitude estimations, $\partial c/\partial x$ can be assumed $(c_{pack} - m \cdot c_{prod})/L$, where c_{pack}, the (constant) concentration of the hazard in the package material ($mg \cdot m^{-3}$); m, the partition coefficient for the package and the product (assumed to be 1); c_{prod}, the concentration in the product (assumed to be 0 $mg \cdot m^{-3}$ initially); L the characteristic length of the product (m). If $c_{prod} << c_{pack}$, the change of c_{prod} in time can be described with zero-order kinetics. In other cases, first-order kinetics can be assumed. In many cases kinetics will show to be of no importance, but in certain cases it will be. So focus may be different, but in principle procedures might be equal.

2.3 Physical and chemical hazard characterisation

For physical hazards, health consequences (for example broken teeth and internal wounds) are generally more likely with increasing size and sharpness of the hazard. For rough risk assessments, an attention value (*AV*) can be used, expressing a threshold size. For example, the USDA guidance concerning hard or sharp foreign objects considers hard or sharp objects that measure between 2 and 7 mm a non-hazardous defect (11), so *AV* for glass and metal can be 2 mm.

Chemical hazards are generally divided into threshold and non-threshold hazards. For threshold hazards, safe levels for human exposure are generally established by the 'no observed adverse effect level' (NOAEL), which is based on animal testing. Subsequently, the NOAEL is divided by a safety factor (usually 100) to account for

possible intra- and interspecies differences, resulting in the 'acceptable daily intake' (ADI) in mg/kg body weight (20). The ADI is comparable to the *AV* in microbiological hazard characterisation.

Non-threshold hazards are for example genotoxic carcinogens and sensitising agents. An *AV* cannot be set for these hazards and therefore the most conservative and simplest model can be used; the one-hit model (20): $P(d) = 1 - \exp(-\beta d)$, which is equal to the exponential model used for microorganisms.

The probability of having problems, given that the product is contaminated, can be expressed by a characteristic number such as the formerly mentioned *HC* (health-problem characterisation). Bacterial levels generally range over many orders of magnitude, so a logarithmic transformation is justifiable. For physical and chemical hazards much smaller ranges will be encountered, therefore a linear scale will often be used.

2.4 Physical and chemical risk characterisation

The probability of having problems, given exposure and the probability of contamination, can be expressed as $PC = HC \cdot OC$. The *PC* value can be used for risk and hazard ranking. Based on the rough risk assessment, risk-determining phenomena can be studied more accurately. More accurate risk characterisation is a logical consequence of more accurate estimation of these phenomena.

2.5 Level 2 analysis

For further analysis of physical hazards in level 2, it is sensible to study removal more accurately, if it is risk-determining. When no removal occurs, the prevalence of contamination is amongst the aspects that can be studied more accurately. More accurate description of the relation between size and probability of problems can be useful if the dose-response relation is risk-determining. Qualitative indications of severity can be used for hazard ranking, and were given for various hazards by the FDA Health Hazard Evaluation Board (11).

For further analysis of chemical hazards in level 2, more accurate study of formation and inactivation of chemicals may be relevant. As for microbiological hazards, this can be done by comparing results of various quantitative models. For example, various models for inactivation of the trypsin inhibitors in soy flour were described and compared by Van den Hout *et al.* (16). As for microbial hazards, support in estimation of contamination levels can be provided by showing contamination data from various references, such as the annual KAP report (18), and O'Keeffe and Kennedy (10).

Table 1. Tentative evaluation of the applicability of the SIEFE model's rough level of detail for chemical and physical hazards. The SIEFE model has a product oriented approach.

Components of QRA	Microbiological	Physical	Chemical
hazard identification			
- *obvious hazards*	•case histories	•case histories	•limited: case histories •generally well known hazards •hazards of public concern
- *other relevant hazards*	•presence in ingredients	•sources of foreign bodies	•surveillance data
exposure assessment			
- *contamination*	•every product •minimum value: 1 CFU·serving^{-1}	•every product	•every product •maximum value: MRL *
- *kinetics*	•growth & inactivation •1st order kinetics	•changes in size	•changes in concentration •1st order kinetics •Fick's law
	•*SC* •*OC*	•*SC* •*OC*	•*SC*
	•logarithmic scale	•linear scale	•linear scale
hazard characterisation	•*AV*: concentration (CFU·serving^{-1})	•*AV*: size (mm)	•threshold: *AV*: ADI † •non-threshold: exponential model
	•*HC*	•*HC*	•*HC*
risk characterisation	•*PC* = *HC* + *OC* (*PC*, *HC*, and *OC* on logarithmic scale)	•*PC* = *HC*·*OC*	•*PC* = *HC*

* MRL: Maximum Residue Level (mg·kg^{-1} food).
† ADI: Acceptable Daily Intake (mg/kg body weight).

In general, for physical and chemical hazards contamination is more relevant than kinetics if compared to microbiological risk assessment. Nevertheless, for specific cases, such as the formation of mutagenic heterocyclic amines on meat surfaces by grilling and roasting, kinetics are also largely relevant, therefore similar approaches can be useful.

3. Concluding remarks: SIEFE, QRA, and food production practice

The SIEFE model is a systematic tool for microbiological quantitative risk assessment for food products. It provides clear quantitative insight into microbial behaviour during production processes, and focuses on the most relevant problems related to food safety.

In this chapter it was shown that the structure and features of the SIEFE model may also be practically relevant for physical and chemical QRA for food products, with supplements and some modifications (Table 1). The availability of quantitative models and the parameters describing the fate of physical and chemical hazards is relevant for QRA in certain cases, but often contamination data are of more relevance. Food chemical risk assessment has gained much research interest, and various approaches for chemical QRA have been developed in past years. Most of these approaches are rather conservative in risk assessment, as is the application of the SIEFE model for chemical hazards, described in this chapter. An important difference is that SIEFE is product-oriented, whereas other approaches are mostly health-effect oriented.

Having a product-oriented approach, and given the gained quantitative insight, and focus on relevant phenomena, the SIEFE model can be used as some sort of member of a HACCP team. It then is a multidisciplinary member in the form of a structured method that couples predictive models, process engineering models, and databases containing qualitative expert and literature knowledge, and quantitative data. Obviously, there are various aspects of the SIEFE model that can be extended or improved in the future. Some examples are:

- For practical use databases and expert knowledge in the SIEFE model need to be extended and continuously updated;
- A procedure to systematically go from the second level to the third level of detail of the SIEFE model has to be developed. The procedure should support the user in structured selection of parameters that need stochastic description; phenomena that need more quantitative information (for example by specific experiments); and phenomena that turned out to be of little quantitative relevance after all.

- The effects of the assumption of homogeneous spatial distribution of the hazards throughout the product needs to be studied. In case of clumping of microbiological hazards, some products will contain high levels of the hazard, whereas others are clean;
- The effects of re-use of product streams during production processes (rework) should be included. This may be an important source of recontamination;
- Inclusion of primary production, and consumer practices will lead to description of the whole production chain; QRA from farm to fork. Inclusion of primary production needs quantitative data and models specially focused on contamination routes;
- Inclusion of quantitative information on toxin formation by toxinogenic pathogens under various conditions will be very helpful for mQRA. During this research project, we did experiments to quantitatively estimate enterotoxin A formation by *Staphylococcus aureus* 196E in relation to growth at various temperatures; in various media; and with/without background flora. The data provide important quantitative information on enterotoxin formation, but have not been included in the SIEFE model, yet. A paper on the study into enterotoxin formation is in preparation;
- An interesting aspect for inclusion in the SIEFE model is germination of spores after heat treatment;
- Biological problems such as moulds that form mycotoxins, flies that carry pathogens, and viruses need to be evaluated;
- Survival models for microorganisms should be included;

Despite the above examples, for microbial food safety control, the present state of the SIEFE model serves decision making well. The main problems can be pinned down efficiently, and non-relevant aspects can be omitted.

The phenomenon mQRA is a scientifically based activity, that is still rather academic. For QRA to be practically relevant, it is necessary that structured, orderly, and easy to use methods become available. In addition, the methods should be clear, and assumptions have to be made explicit. The methods should highlight risk-determining factors, and compare the (relative) effects of management options. Implementation of these methods as expert systems, such as the SIEFE model, will probably serve the process towards realisation of QRA in food production practice. Since QRA is an emerging area or research it is likely that the near future will bring more methods and expert systems.

The practical applicability of quantitative risk assessment will increase enormously if quantitative data are readily available. Since people all over the world benefit from optimal food safety control, it is sensible to continue international co-operation in

this field. The ICMSF book 'Microorganisms in foods, volume 5' (5) is a good example of extensive data acquisition beyond organisation and national borders. The development of standardised databases on known foodborne outbreaks, pathogen characteristics, prevalence and levels of contamination, and presence in (industrial) environments should be the next step.

For various sorts of quantitative data, data acquisition actually means filling gaps in knowledge. The most obvious lacuna is the lack of knowledge on quantitative dose-response relations in humans. Prevalence of (re)contamination under various conditions is another example. This can often only be quantified by rough guesses. Quantitative risk assessment models, such as the SIEFE model, can be used to efficiently allocate research needs.

For now, it is sensible to make best use of the information that is available, notifying its shortcomings. As application of the SIEFE model showed, lack of quantitative data does not necessarily prevent quantitative risk assessment. Worst-case estimates can be sufficient for some risk-determining phenomena, whereas other risk-determining phenomena can be described by a range of values. Using plausible ranges for parameters actually is some sort of sensitivity analysis, providing insight into truly relevant aspects. Still, quantitative data are crucial for quantitative risk assessment, and therefore international research projects and open data exchange are the risk assessor's best friends.

QRA will help regulatory agencies in setting quantitative criteria related to food safety, the so called food safety objectives, by increased quantitative insight into production processes. Given a food safety objective, QRA will help food producers by easy evaluation of different conditions of, for example processing, and consumer practices (9,19). Yet, the possible applications of QRA go much further. Dynamic programming (DP) can be used to optimise processes according to a certain risk, or food safety objective. For example, given a food safety objective, DP can help designing processes such that quality losses, or costs are minimal.

For whatever goal QRA is used, it is important that virtues and shortcomings of the risk estimates are well defined to prevent misinterpretation. In our opinion, striving for very accurate risk estimates will not be as useful for food safety control as striving for quantitative insight into production processes.

As D.R. Tennant (15) concluded: '*We will never have a complete set of perfect tools, since the underlying physical, biological and social sciences will continue to evolve. We do, however, have a responsibility to make the best use of the tools which are available now so that we can identify the best possible solutions, not the most obvious*'. A necessary condition for making the best use of the available tools is trans-

parency, since transparent risk assessments are open for criticism, and adjustments if new information becomes available. As mentioned by Zwietering and Van Gerwen (22): '*This criticism should not be used to condemn the analysis, but to improve the results*'. To our opinion, transparent risk assessment can best be achieved by a stepwise approach. A simple start, and determination of essential and negligible aspects before going into detail gives the best insight into the complex field of risk assessment. The stepwise approach is a powerful tool in decision making, endorsing the strength of simplicity.

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Summary

Food safety is a prerequisite for food products, since consumers trust on buying safe foods. Food safety management systems, such as the Hazard Analysis Critical Control Point (HACCP) system, have gained much interest in the past years. Momentary, food safety is often managed for a large part on qualitative grounds. A quantitative approach of food safety management is useful by objective analyses, and can be attained by quantitative risk assessment (QRA). This thesis describes a method for stepwise microbial QRA for food products in general and various steps of QRA.

The first step in QRA is hazard identification, which is qualitative. An identification procedure for foodborne microbial hazards has been developed, and implemented as an expert system. The procedure is product-oriented; it selects microbial hazards related to a specific food product. The hazard identification consists of three levels of detail. First, it selects the most obvious hazards for a product, based on reported foodborne outbreaks in the past. Second, in more detailed analysis, hazards are selected based on reported presence of pathogens in the ingredients of the product. Finally, comprehensive hazard identification can be performed for selection of unexpected hazards. In case of selection of many hazards, knowledge rules support the user in selecting the most relevant hazards for a product, making the procedure interactive.

After hazard identification, exposure assessment is performed as part of QRA. Modelling microbial growth and inactivation is an important aspect of exposure assessment. Many predictive models have been developed in the past years, varying from general and simple models to specific and advanced models. Since no model is able to accurately predict microbial responses under all conditions, it is sensible to start with simple models and obtain order of magnitude estimates. If relevant, more accurate estimates can best be gained by comparing various models. It was shown that advanced models not necessary result in better estimates. In other words, the virtues of simple models were shown for both rough and detailed exposure assessments.

Estimation of the extent of inactivation under various conditions is also part of exposure assessment. For inactivation by irradiation, we studied the quantitatively most relevant factors for the irradiation parameter D_{10}. A data analysis of 539 D_{10} values from the literature resulted in a first classification of D_{10} in spores and vegetative bacteria, with spores having significantly higher D_{10} values. Further analysis con-

firmed extreme high resistance of various vegetative bacteria. The categorisation of quantitatively important factors into separate D_{10} categories is a useful tool in designing and evaluating irradiation processes.

Next to hazard identification and exposure assessment, hazard characterisation and risk characterisation are the third and fourth aspects of QRA. These four aspects have been integrated in a stepwise approach for QRA; the SIEFE model. SIEFE is an acronym for Stepwise and Interactive Evaluation of Food safety by an Expert system. The main goal of the SIEFE model is obtaining quantitative insight into food production processes. The stepwise approach starts roughly and semi-quantitatively, to find risk-determining phenomena. These phenomena can then be studied more accurately in a second level of detail. Non-relevant aspects can be omitted in this level, simplifying the complex problem of microbial food safety assessment. A third level of detail can be used for even more detailed analyses, for example stochastic description of parameters.

The SIEFE model providing quantitative insight into food production processes has been shown by application of the SIEFE model to two example products. In addition, this was confirmed by a comparison of the SIEFE model to another approach of microbial QRA from the literature. Transparent risk assessment was shown to be a powerful tool in decision-making, even if not all necessary quantitative information is available.

Samenvatting

Voedselveiligheid is een randvoorwaarde bij het produceren van levensmiddelen. De consument vertrouwt er op dat hij veilig voedsel koopt. Vanwege het belang van veilig voedsel staan kwaliteitsborgingssystemen volop in de belangstelling. Een bekend systeem is bijvoorbeeld het Hazard Analysis Critical Control Point (HACCP) systeem. De huidige systemen zijn voornamelijk kwalitatief van aard. Een kwantitatieve benadering is een nuttige aanvulling omdat dit een objectieve analyse van de processtappen mogelijk maakt. De objectieve analyse kan worden uitgevoerd met behulp van kwantitatieve risicobepaling (Quantitative Risk Assessment, QRA). Dit proefschrift beschrijft een methode voor QRA voor levensmiddelen, welke zich richt op microbiologische gevaren. Hierbij zijn een aantal onderdelen van QRA in detail uitgewerkt en beschreven.

Het eerste onderdeel is de gevarenidentificatie, welke kwalitatief van aard is. Er is een procedure voor gevarenidentificatie ontwikkeld, die uitgaat van het product; voor een specifiek levensmiddel worden de gevaren geselecteerd. De stapsgewijze procedure, geïmplementeerd in een computerprogramma, selecteert als eerste stap de meest voor de hand liggende gevaren voor een product. Deze gevaren zijn de pathogenen, waarvan gerapporteerd is dat ze in het verleden tot problemen hebben geleid in het product. Een tweede stap is meer gedetailleerd, waarbij pathogenen worden geselecteerd die aanwezig zijn in de ingrediënten en zodanig tot problemen kunnen leiden in de toekomst. De derde stap is een allesomvattende gevarenidentificatie, voor selectie van onvoorziene gevaren. Wanneer de selectie resulteert in een lange lijst van gevaren, bieden kennisregels hulp bij het selecteren van de meest relevante gevaren. Door het gebruik van de kennisregels heeft de procedure een interactief karakter.

Het schatten van de blootstelling van de consument aan het gevaar is het volgende onderdeel van QRA. Modellen voor microbiële groei en inactivatie spelen hierbij een belangrijke rol. In de loop van de jaren zijn er vele modellen ontwikkeld, variërend van simpele, algemeen bruikbare modellen tot uitgebreide, specifieke modellen. Tot op heden is er geen eenduidigheid over welk model het best voorspelt onder bepaalde omstandigheden. Voor het verkrijgen van grootteorde-schattingen is er daarom gekozen voor het gebruik van de meest eenvoudige en praktische modellen. Indien relevant kunnen groei en/of inactivatie vervolgens nauwkeuriger

bestudeerd worden, door diverse modellen met elkaar te vergelijken. Hierbij is gebleken dat uitgebreide modellen niet per se tot betere schattingen leiden, en dat eenvoudige modellen in zowel grove als gedetailleerde risicobepalingen een belangrijke bijdrage kunnen leveren.

Het schatten van de mate van inactivatie ('afdoding') van pathogenen onder variërende omstandigheden is een onderdeel van de blootstellingsbepaling. Voor inactivatieprocessen door middel van doorstraling werden de kwantitatief meest belangrijke factoren voor de doorstralings-parameter, D_{10} (min) bestudeerd. Een data-analyse van 539 D_{10} waarden uit de literatuur heeft geresulteerd in een eerste indeling in factoren die een belangrijke invloed hebben op D_{10}. Deze indeling is in sporen en vegetatieve cellen, met sporen als significant resistenter dan vegetatieve cellen. Bij verdere analyses kwam naar voren dat een aantal soorten vegetatieve bacteriën extreem resistent zijn voor doorstraling. De indeling van invloedsfactoren in verschillende D_{10}-categorieën is een nuttige leidraad bij het ontwikkelen en evalueren van doorstralingsprocessen.

QRA bestaat naast de hiervoor genoemde gevarenidentificatie en blootstellingsbepaling uit gevarenkarakterisering en risicokarakterisering. Deze vier onderdelen zijn tesamen geïntegreerd in een methode voor microbiële QRA, het SIEFE model. SIEFE is een acronym voor Stepwise and Interactive Evaluation of Food safety by an Expert system. Het belangrijkste doel van het SIEFE model is het verschaffen van kwantitatief inzicht in productieprocessen van levensmiddelen. De methode is stapsgewijs; in eerste instantie worden vrij grove risicobepalingen uitgevoerd voordat er in detail getreden wordt. De grove analyse legt de vinger op de gevoelige plekken in het productieproces. In een volgende analyse worden deze plekken nauwkeuriger bestudeerd, en kunnen niet-relevante aspecten overgeslagen worden. Dit laatste leidt tot een aanzienlijke vereenvoudiging van het complexe probleem van microbiologische voedselveiligheid. Indien relevant kunnen risicobepalende aspecten hierna nog verder onderzocht worden, bijvoorbeeld door stochastische analyses.

Dat het SIEFE model kwantitatief inzicht levert is gebleken uit de toepassing van het SIEFE model op twee voorbeeldproducten. Bovendien heeft een vergelijking van het SIEFE model met een benadering van QRA uit de literatuur het bereiken van het doel bevestigd. Het SIEFE model toont hiermee dat kwantitatieve risicobepaling, waarvan alle stappen en aannames helder omschreven zijn, een belangrijk instrument kan zijn bij beslissingen over voedselveiligheid, zelfs als niet alle benodigde kwantitatieve informatie beschikbaar is.

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Nawoord

"Ik word noooooit AIO !" Degene die het hardst roept is er vaak snel bij, zo bleek maar weer. De keuze om te promoveren is een hele goede geweest en aan het plezierige verloop van de afgelopen jaren hebben een aantal mensen in belangrijke mate bijgedragen.

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Curriculum vitae

Suzanne van Gerwen werd op 7 september 1971 geboren te Beek en Donk. In 1989 behaalde zij het VWO diploma aan het Dr. Knippenbergcollege te Helmond. In datzelfde jaar begon zij met de studie Levensmiddelentechnologie aan de Landbouwuniversiteit in Wageningen. Afstudeervakken werden afgelegd in de richtingen Levensmiddelenproceskunde en Kwaliteitsborging van levensmiddelen. Dit laatste vak werd uitgevoerd bij Red Band-Venco in Roosendaal. De studie werd afgerond met een stage aan de Chemical Engineering Department, Adelaide University in Adelaide, Australië. In juni 1995 studeerde zij af.

Vanaf juli 1995 tot en met juli 1999 werkte zij aan haar promotieonderzoek bij de sectie Proceskunde, Landbouwuniversiteit Wageningen. De resultaten staan beschreven in dit proefschrift.

Vanaf oktober 1999 is zij werkzaam bij Unilever Research Vlaardingen, in de unit Microbiology and Preservation.