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# Quantitative microbial risk assessment exemplified by *Staphylococcus aureus* in unripened cheese made from raw milk

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## Abstract

This paper discusses some of the developments and problems in the field of quantitative microbial risk assessment, especially exposure assessment and probabilistic risk assessment models. To illustrate some of the topics, an initial risk assessment was presented, in which predictive microbiology and survey data were combined with probabilistic modelling to simulate the level of *Staphylococcus aureus* in unripened cheese made from raw milk at the time of consumption. Due to limited data and absence of dose–response models, a complete risk assessment was not possible. Instead, the final level of bacteria was used as a proxy for the potential enterotoxin level, and thus the potential for causing illness. The assessment endpoint selected for evaluation was the probability that a cheese contained at least 6 log cfu *S. aureus* g<sup>-1</sup> at the time of consumption; the probability of an unsatisfactory cheese,  $P_{uc}$ . The initial level of *S. aureus*, followed by storage temperature had the largest influence on  $P_{uc}$  at the two pH-values investigated.  $P_{uc}$  decreased with decreasing pH and was up to a factor of 30 lower in low pH cheeses due to a slower growth rate. Of the model assumptions examined, i.e. the proportion of enterotoxigenic strains, the level of *S. aureus* in non-detect cheeses, the temperature limit for toxin production, and the magnitude and variability of the threshold for an unsatisfactory cheese, it was the latter that had the greatest impact on  $P_{uc}$ . The uncertainty introduced by this assumption was in most cases less than a factor of 36, the same order of magnitude as the maximum variability due to pH. Several data gaps were identified and suggestions were made to improve the initial risk assessment, which is valid only to the extent that the limited data reflected the true conditions and that the assumptions made were valid. Despite the limitations, a quantitative approach was useful to gain insights and to evaluate several factors that influence the potential risk and to make some inferences with relevance to risk management. For instance, the possible effect of using starter cultures in the cheese making process to improve the safety of these products.

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## 1. Introduction

Risk analysis within the field of food safety is a strongly evolving activity and during recent years several meetings have addressed the details of risk management (FAO/WHO, 1997), risk assessment

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(CAC, 1999) and risk communication (FAO/WHO, 1998). Since the latest Food Micro meeting 1999 in Veldhoven, which hosted a session devoted to risk assessment the WHO/FAO and CODEX have launched several risk assessments for different pathogen–food combinations, which have been discussed in a series of expert consultations (e.g. FAO/WHO, 2000, *in press*). The purpose of developing these assessments has been to address specific risk management questions and to improve and disseminate the methodology for risk assessment. Another important objective of these activities was to develop guidelines and good practices for the conduct of the different steps involved in a quantitative risk assessment, e.g. hazard characterisation and dose–response (FAO/WHO, *in preparation(a)*), and exposure assessment (FAO/WHO, *in preparation(b)*). It can be envisaged that when these documents have been finalised most food safety issues will be addressed by development of quantitative risk assessment models at the international level. The models will have to be adopted to reflect the conditions at the national level. In this respect it may be anticipated that the exposure assessment will be of particular importance due to national differences in how food is produced and distributed, in the occurrence of specific microorganisms, and in the way food is prepared and consumed.

Risk is defined as the probability and the consequences of a hazard to occur. A risk in the context of food safety is the probability and the consequences of adverse health effects following the ingestion of food. The separation of risk into two components is useful, since risk may be managed both by actions to reduce the probability and the consequences of the adverse event. The second component is often overlooked in microbial risk assessments, although it may implicitly be considered in the selection of the biological endpoint in the dose–response relationship, e.g. diarrhoea, morbidity, mortality. However, to be able to compare the public health risk and benefits of different management options, it would be desirable to be able to integrate different biological endpoints in one public health measure. One such measure is Disability Adjusted Life Years (DALY), which is the sum of years of life lost by premature mortality and years lived with disability, weighted with a factor between 0 and 1 for the severity of illness (Murray, 1996). For instance, Havelaar et al. (2000a,b) reported that the

more infrequent consequences such as gastroenteritis related mortality (310 DALY) and Guillain–Barre syndrome (340 DALY), contributed as much as frequent acute gastroenteritis (440 DALY) to the total health burden of *Campylobacter* associated illness in the Dutch population per year (1440 DALY).

Risk analysis consists of three partly overlapping components; risk management, risk assessment, and risk communication, and can be described as a framework to analyse and manage any activity that may have negative consequences. In the context of food safety, it is a tool, which in a formalised, systematic and transparent way, enables responsible authorities and international organisations to understand and if necessary evaluate options to reduce a health risk. Risk assessment is a science-based process in which questions that have been formulated during the risk evaluation step of the risk management process are addressed to develop an understanding of the problem and to come up with risk estimates. Reviews of the four steps in a formal risk assessment were presented at the latest Food Micro meeting in Veldhoven 1999; hazard identification and exposure assessment (Lammerding and Fazil, 2000) and hazard characterisation and risk characterisation (Buchanan et al., 2000), respectively. In short, in the exposure assessment, the likelihood that an individual or a population will be exposed to a microbial hazard and the likely numbers ingested are estimated (Lammerding and Fazil, 2000). In the hazard identification step, the relevant hazards, i.e. microorganisms and/or their toxin(s), and their main sources together with the relevant context are described. The likelihood of a response of any individual to an exposure to a foodborne pathogen is dependent on the integration of host, pathogen and food matrix effects which are described in the hazard characterisation step (Buchanan et al., 2000). In the final risk characterisation step, the exposure assessment serves as the input to the dose–response model selected from the hazard characterisation, in order to evaluate the risk in relation to the risk management question. An important part of this step is to estimate the uncertainty of the estimates and the impact of critical assumptions made in the assessment (Buchanan et al., 2000).

The purpose of the present work is to discuss some of the developments and problems in the field of quantitative microbial risk assessment, especially as

regards exposure assessment and probabilistic risk assessment models. As an illustration of some of the topics, an initial risk assessment of *Staphylococcus aureus* in unripened cheese made from raw milk is presented.

## 2. Selected topics in probabilistic exposure assessment

The Veldhoven reviews (Buchanan et al., 2000; Lammerding and Fazil, 2000) discussed the advantages of probabilistic modelling for addressing variability and uncertainty of parameters in a risk assessment model. The majority of risk assessments use a probabilistic approach but it may be considered good practice to begin with a more simple deterministic model to explore if probabilistic models are necessary (EPA, 1997; Zwietering and van Gerwen, 2000). Probabilistic techniques, such as Monte Carlo analysis (Vose, 2000), have been demonstrated to be useful tools for analysing the variability and uncertainty associated with model parameters in quantitative risk assessments. Uncertainty, which refers to a lack of knowledge, can be reduced by further study and includes (EPA, 1997): (1) Scenario uncertainty (descriptive errors, aggregation errors, errors in professional judgement, incomplete analysis); (2) model uncertainty (uncertainty due to necessary simplification of real-world processes, mis-specification of the model structure, model misuse, use of inappropriate surrogate variables); and (3) parameter uncertainty (measurement errors, sampling errors, systematic errors). Variability refers to the natural variation in the system under study, and further studies can lead to a better characterisation of variability, but a change of the system, e.g. changing the food production system, is needed to reduce it.

In a probabilistic model, each uncertain model input parameter is described by probability distributions rather than by point estimates. There are a number of techniques to calculate the outcome distribution such as the method of moments, exact algebraic solutions and Monte Carlo simulation (Vose, 2000). Using the Monte Carlo approach, the model is calculated a number of times to simulate the outcome distribution. Each time (iteration) the model is calculated values for the model parameters are sampled

from the probability distribution defined for the parameters, and represent, in principle, a scenario or event that may occur. The input distributions are sampled at random using the Monte Carlo or more often the Latin Hypercube, LHS, sampling methods. LHS is a form of stratified sampling method often regarded superior to the Monte Carlo method since it reproduces the input distributions in fewer iterations (Vose, 2000). However, LHS may place less emphasis on sampling from the tails of the input distributions and may be hard to implement in hierarchical model structures consisting of several sub-models (Jordan et al., 1999). The simulation result is a frequency distribution of the output of interest which provide not only extreme values but also the most likely outcome based on the combinations of input probability values that could occur. The utility of probabilistic techniques is dependent on the availability of adequate data, credible assumptions, and application of good scientific practices in terms of clarity, transparency, reproducibility, and sound use of methods (EPA, 1997). In the field of quantitative microbial risk assessment, the approaches to these issues and sound use of methods are currently being developed. In this section, some considerations related to the scope and development of exposure assessments of food-borne microbial hazards will be addressed. The present description is by necessity sequential and ordered linearly along a time axis, in reality though the risk assessment process is dynamic and iterative in nature and many of the steps may be iterated and/or carried out in parallel (Anonymous, 2000).

### 2.1. The purpose of the risk assessment

The purpose and objective of an assessment should guide its conduct (Morgan and Henrion, 1990) and, in order to clearly and concisely define these, a close interaction between managers and assessors is necessary during the initial phases. In some instances it may be necessary to limit the scope to be able to address the questions by making them more specific or, alternatively, to develop more than one assessment. The exposure assessment should be made as simple as possible while still including the important sources of risk.

Based on the outcome of this initial phase decisions regarding the approaches to modelling, e.g.

probabilistic or deterministic, dynamic or static, empirical or mechanistic, and the structure of the assessment model (which pathways, single or multiple models) can be made. Dynamic models describe a process over time (or space) and are often constructed in terms of differential or difference equations, which describe the rate of change. In contrast, static models consider the probability of an event happening during a given time period or at a point in time (or location). Most QMRA's have been driven by static risk management questions and the estimation of risk can usually be termed static, although they may contain elements of dynamic modelling as well, e.g. modelling of microbial growth.

## 2.2. Monte Carlo simulation and the separation of uncertainty and variability

The basic goal of a Monte Carlo analysis is to quantitatively characterise the uncertainty and variability in terms of risk, and to identify and understand the relative contribution of the key sources of this uncertainty and variability. There may be instances where a probabilistic Monte Carlo analysis for various reasons is not an option. For instance, when it is not expected to improve a risk assessment, when the risk is well below concern, when neither time nor resources are available, when the problem can be managed at a low cost anyway, or when rare events have a large impact on the risk (EPA, 1997). In many other situations, probabilistic Monte Carlo analysis may be useful, e.g. when conservative point estimates fall above levels of concern, in order to rank exposure sources, exposure pathways or contaminants, or when costs are high or the consequences of not managing the problem are unacceptable. In practice, a tiered approach beginning with a simple screening model and progressing to more sophisticated and realistic models may often be the preferred approach (EPA, 1997).

The separation of variability and uncertainty of parameters in QMRA models, second-order models, have up to now rarely been made, a reflection of the fact that this can be a daunting task. However, neglecting the difference between them may lead to improper risk estimates (Nauta, 2000) and/or incomplete understanding of the results (Vose, 2000). Also, if the distinction is not clear to the analyst, a

variability distribution may incorrectly be used as if it were an uncertainty distribution (Vose, 2000). The explicit separation of these two allows decision-makers to understand how model outputs might improve if uncertainty is reduced. There are essentially two methods for producing second-order models; the first calculates variability and simulates the uncertainty, and the second method simulates the variability, selecting for each simulation a random sample from distributions for uncertain parameters (Vose, 2000).

## 2.3. The pathways and steps included in the exposure assessment

The relevant stages of the farm-to-fork chain, the key processes and the level of detail necessary to estimate the probability and the likely levels of exposures are determined with reference to the assessment end point as defined by the risk management question. It may not be possible or even necessary to model the whole farm-to-fork pathway or every conceivable event in a system that may have an impact on the exposure. Depending on the emphasis and the perspective of the risk assessment, different approaches have been used in developing the overall model. For instance, the Event Tree describes a scenario from the initiating event to a defined endpoint of the assessment (Roberts et al., 1995). This approach serves to describe the high-risk pathways that lead to contamination and subsequent disease and may identify risk variables in need of further data or modelling. In contrast to the Event Tree, the Fault Tree begins with the occurrence of a hazard and from there describes the events that must have occurred for the hazard to be present (Roberts et al., 1995). This approach can provide a framework to analyse the likelihood of an event by determining the complete set of underlying conditions or events that allow the given event to occur (Jaykus, 1996). Additional approaches to modelling used in assessments of microbial food hazards include a Dynamic Flow Tree model (Marks et al., 1998) and a Process Risk Model (Cassin et al., 1998). The former emphasises the dynamic nature of bacterial growth and incorporates predictive microbiology using statistical analysis of data, whereas the latter focuses on the integration of predictive microbiology and scenario analysis

to provide an assessment of the hygienic characteristics of a manufacturing process. Variations on these themes exist. More elaborate and sophisticated classifications and distinctions between approaches and types of models than in the present work have been proposed (see e.g. Hurd and Kaneene, 1993). The broad types of models described here operate in only one direction, which does not make the inclusion of feedback mechanisms possible. This may be a limiting factor when modelling complex biological systems. Alternative models may include dynamic models based on differential equations, or Markov chain, and random-walk models or so-called neural networks (Skjerve, 1999). The use of alternative structural and mathematical models, together with various types of model errors can represent important sources of uncertainty. It should be noticed that methods for dealing with uncertainty associated with the choice of the structure of risk models are lacking (Morgan and Henrion, 1990). Preliminary analysis using alternative structural models may be examined to determine if structural differences have important effects on the outputs of the model. However, the scenario or event being modelled must be kept in mind to ensure that the model provide answers to the questions posed, that an event that could physically occur in reality is described, and that comparable data which can be combined in the model are used (Vose, 2000).

Since the exposure model itself is a tool to understand the problem under study and to identify knowledge gaps, it is desirable that it is developed independently from the consideration of the availability of data. This may be difficult in practice, since the choice of model may be very dependent on the data that is available (Nauta, 2001). The data to feed into probabilistic risk assessment models need to be compiled and critically evaluated in terms of sampling and analytical errors. The lack of representative data is a major threat to the accuracy of a variability analysis. For instance, data from a survey may have to be weighted to correct for biases in the sampling, e.g. based on the annual production in different regions (e.g. Ebel and Schlosser, 2000). Further, data from different sources that were collected over different temporal or spatial scales may be the only data available and the direct combination of these may be difficult. In addition, the estimates

may be biased due to differences in the methodology used, e.g. in terms of sensitivity and specificity. If data on these are collected or can be calculated based on the survey results, apparent estimates can be adjusted to come up with true prevalence estimates (Ebel and Schlosser, 2000).

If the primary goal is to estimate the risk to a population from a food–pathogen combination, it may suffice to structure the model as to use data and information as close to the consumption point as possible (e.g. Lindqvist and Westöö, 2000). This approach could be useful for risk ranking as well (e.g. US-FDA/FSIS, 2000). In contrast, this approach has a more limited application in gaining insights into the factors magnifying the risk or for consideration of options to reduce the risk.

Given the complexity of many interrelated processes in any selected food pathway, it is often necessary to separate the overall pathway into a number of distinct modules each representing a particular stage from production to consumption (Lammerding and Fazil, 2000). Common processes and stages to be modelled in the exposure assessment can be identified. These include production, distribution, storage, processing, preparation and handling. This commonality opens up the possibility to develop common approaches for performing exposure assessments. In the future, libraries or clearinghouses can be envisioned which contains common exposure assessment sub-modules that may be modified to the specific problem by the potential end-user.

A general framework for quantitative exposure assessment modelling, the Modular Process Risk Model (MPRM), was recently proposed (Nauta, 2001). In this framework, the consumption stage was not addressed and processes in the primary production were not explicitly considered. The framework resembles the approach of the Process Risk Model (Cassin et al., 1998), but at the heart of the proposal is the suggestion that each of the process steps in the exposure assessment can be identified as one of six basic processes; growth, inactivation, partitioning, mixing, removal and cross-contamination. In each step, the number of microorganisms per unit, e.g. carcass, bottle of milk, a package of ground beef, is estimated. The size of the unit may change in the food pathway being described, for instance as a result of mixing, i.e. several units are

blended into a larger unit, or partitioning, i.e. a unit is separated into smaller units. Removal is the process when some units are selected and removed from the process. Each of the six basic processes describes a specific sub-module in the model, and for each basic process a variety of models can be applied. While theoretically possible to allocate a basic process to each processing step, this may, in some cases, be unnecessary or too complex in relation to the purpose of the exposure assessment. In those cases, it was proposed that consecutive processes could be considered together, aggregated, and described by a “black box” model. In general, a model should be broken down into smaller components, disaggregated, as much as necessary but not more for an efficient but accurate modelling in relation to the purpose of the assessment (Vose, 2000).

A beneficial consequence of the development of the risk analysis framework may be the increased use of quantitative approaches to address food safety problems also at the national level. Such an example is presented below.

### 3. Example: initial risk assessment of *S. aureus* in unripened cheese made from raw milk

#### 3.1. Purpose of assessment/hazard identification

Since December 1998, the sale of dairy products produced from raw milk is allowed by derogation in Sweden, if produced and sold on the spot from small-scale facilities. To get a better basis for a review of this derogation regulation an assessment of the risk and the evaluation of some of the factors that influence its magnitude were requested. The biological endpoint to be assessed was acute illness due to consumption of unripened cheese made from raw milk containing toxinogenic *S. aureus*. The presence of *S. aureus* in cheese made from raw milk is a known health hazard (Tham et al., 1990), and outbreaks due to consumption of cheese made from both pasteurised and raw milk have been reported (Bone et al., 1989; de Buyser et al., 2001). The risk was assessed based on data on the prevalence and levels of *S. aureus* detected in these products at the time of sale, and the predicted change in numbers during storage in the homes of the consumers (Fig. 1).

#### 3.2. Hazard characterisation

The acute effects follow after ingestion of pre-formed Staphylococcal enterotoxins (SET) after a short incubation period (1 to 7 h) and include nausea, vomiting, abdominal pain, and diarrhoea (ICMSF, 1996). Not all strains are capable of producing SET, but among the enterotoxigenic strains seven types of toxins have been distinguished based on their antigenic properties. Types A and D are the most common SET's involved in food poisoning. These SET's are formed during the exponential growth of the bacteria, whereas the other types are predominately formed when the bacteria enter the stationary phase (ICMSF, 1996). The existence of long-term effects following a *S. aureus* food poisoning is to our knowledge not established. In animal studies a certain degree of immunity have been shown after repeated exposure to the same type of SET (ICMSF, 1996).

No dose–response relationships were found in the literature. Another limitation is that only data on the number of *S. aureus* bacteria, not the amount of toxin per gram of cheese, was available for this study. SET's have been detected in food containing around  $10^6$  cfu  $g^{-1}$  (Anunciacao et al., 1995), but there are reports when both lower (Rörvik and Granum, 1996) and substantially higher levels (Gockler et al., 1988; Otero et al., 1988) have been required for SET detection. Thus, there are no simple relationships available between the number of bacteria and the concentration of the toxins. This taken together represents substantial knowledge gaps.

#### 3.3. Exposure assessment

##### 3.3.1. Prevalence and level of *S. aureus* in unripened cheese at the time of sale

A survey of enterotoxigenic *S. aureus* in unripened cheese ( $n=37$ ) produced in small-scale facilities in Sweden between August and September 1997 (Sylvén, 1998) indicated that about 30% of the samples contained *S. aureus* above the theoretical detection limit of the analytical method, 100 cfu  $g^{-1}$  (Table 1). Considering the purpose of the assessment and the limited data available, it was decided to treat unripened cheeses made from raw milk as one group. This was a simplification since these types of cheeses include cheeses produced from goats or cows milk,

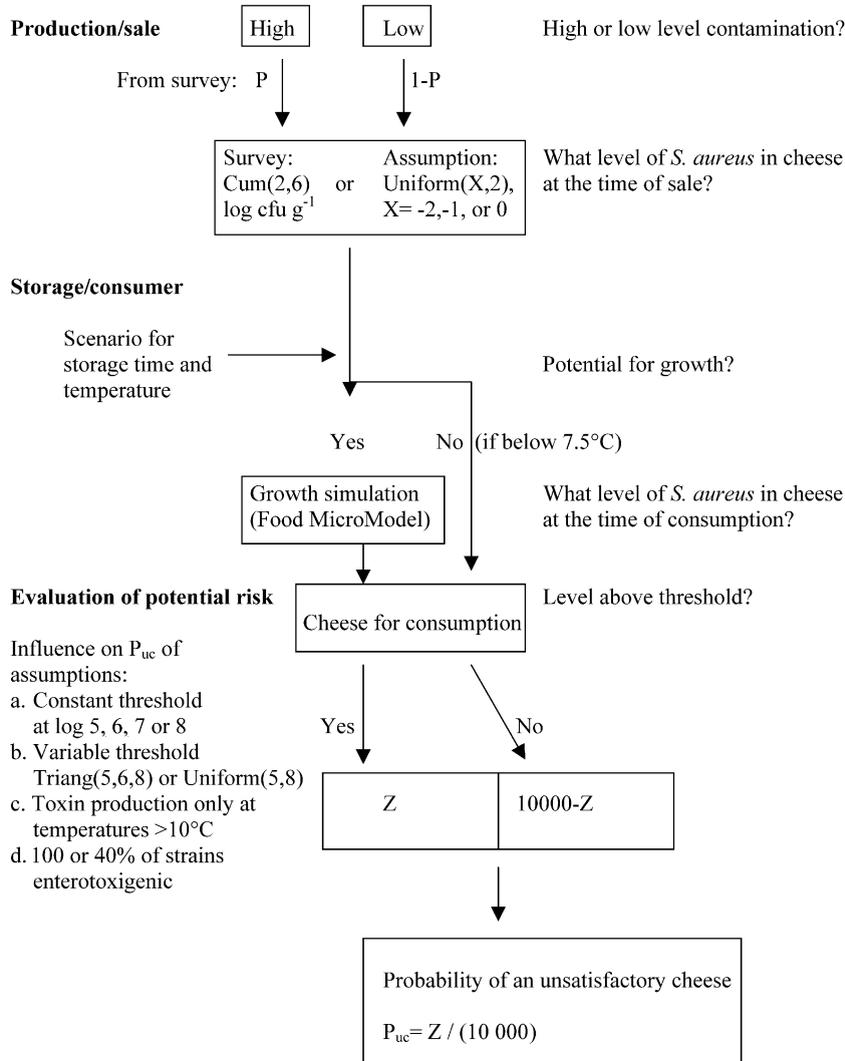
**Step/comments**

Fig. 1. Schematic diagram of model simulating the probability of an unsatisfactory cheese,  $P_{uc}$ . The input parameters of the model are described in Table 2. One simulation consisted of 10,000 iterations and  $Z$  is the number of iterations (cheeses) above the threshold.

and represent different geographical regions and manufacturing processes. When more data becomes available this part of the analysis should be refined. Further, it was assumed that cheeses can be grouped into low level (<100 cfu g<sup>-1</sup>), or high level (>100 cfu g<sup>-1</sup>) cheeses. The distribution of *S. aureus* in low level cheese was assumed to vary uniformly between -1 and 2 log cfu g<sup>-1</sup>, and in high level cheese according to a cumulative distribution as defined by

the results of the survey (Table 2). Seven of the isolated strains were characterised and found to be able to produce SET, although toxin could not be detected in any of the cheese samples analysed.

### 3.3.2. Prevalence and level of *S. aureus* in unripened cheese at the time of consumption

In the absence of data on the level of *S. aureus* in cheese at the time of consumption, these were simu-

Table 1  
Survey of the prevalence and levels of *S. aureus* in unripened cheese produced from raw milk in Sweden 1997 (Sylvén, 1998)

Level (log cfu g <sup>-1</sup> )	No. of samples
Nd (<2)	26
2.8	1
3.0	1
3.3	1
3.6	1
>4.5	3
4.6	1
4.7	1
5.1	1
>5.5	1
Mean <sup>a</sup> : 1.6 log cfu g <sup>-1</sup>	Worst case: 6.0 log cfu g <sup>-1</sup>

*S. aureus* was detected in 11 out of 37 samples.

Nd=not detected.

<sup>a</sup> Mean of the logs. The level in non-detect cheeses was assumed to be 0.5 log cfu g<sup>-1</sup>, i.e. the mean of the Uniform(-1,2) distribution.

lated using a predictive growth model (Food Micro-Model ver. 3.02, Leatherhead, Surrey, UK). Cheeses analysed in the survey were sampled from the cheeses

on display at the producers, and the survey was expected to reflect the levels found at the time of sale. Changes in the levels of bacteria between sale and consumption are a function of inactivation (death) and growth during storage in the refrigerators of the consumers. The storage scenario investigated (Table 2) was based on expert opinions of the producers and the authorities. The predicted rate of inactivation was slow even in the most acid cheeses (pH 4.9), 24 to 55 days for a one-log reduction, compared to the maximum storage time (7 days), and inactivation was therefore not considered.

Predictive models describing growth of *S. aureus* as a function of temperature and pH are available in both the Pathogen Modeling Program (USDA, 1998), and in Food MicroModel, but these cannot be linked directly in an iterative process, which is necessary to perform the Monte Carlo simulation. Therefore, Food MicroModel was used to predict doubling times at storage temperatures between 7.5 and 13.5 °C, and at two pH-values, 5.2 and 6.5, respectively. Growth was assumed to be negligible below 7.5 °C. The data was

Table 2  
Description of model inputs

Symbol	Name	Unit	Assumption/distribution
<i>Producer</i>			
$P_p$	Prevalence of <i>S. aureus</i> in cheeses		0.3 or Beta(11+1, 37-11+1)
$1-P_p$	Prevalence of non-detect cheeses		
$C_{pos}$	Level in positive cheese	Log cfu g <sup>-1</sup>	Cumulative based on data in Table 1 RiskCumul(2;6; {2.8;3;3.3;3.6;4.5;4.6;4.7;5.1;5.5}; {0.083;0.167;0.25;0.333;0.583;0.667;0.75;0.833;0.917})
$C_{neg}$	Level in non-detect cheese	Log cfu g <sup>-1</sup>	Uniform(-1,2), Uniform(0,2) or Uniform(-2,2)
$N_0$	Level of <i>S. aureus</i> in cheese at the time of sale	Log cfu g <sup>-1</sup>	Discrete( $C_{pos}$ : $C_{neg}$ , $P_p$ : (1- $P_p$ ))
<i>Consumer</i>			
$T$	Storage temperature	°C	Trigen(4, 8, 12, 2.5%, 99%) <sup>a</sup>
$t_s$	Storage time	h	Triang(1,72,168) <sup>a</sup>
$t_{lag}$	lag phase at pH 5.2	h	$\ln t_{lag} = -3.82545 \times \ln T + 13.18789$ ( $R^2=0.997$ )
$t_{lag}$	lag phase at pH 6.5	h	$\ln t_{lag} = -3.43821 \times \ln T + 11.53728$ ( $R^2=0.999$ )
DT	Doubling time at pH 5.2	h	$\ln DT = -2.66466 \times \ln T + 9.15909$ ( $R^2=0.998$ )
DT	Doubling time at pH 6.5	h	$\ln DT = -2.51334 \times \ln T + 7.98642$ ( $R^2=0.997$ )
$t_{eff}$	Time for growth	h	$t_{eff} = t_s - t_{lag}$
$N_t$	Level of <i>S. aureus</i> at the time of consumption, $t_s$	Log cfu g <sup>-1</sup>	$N_t = N_0 + \text{Log}_{10}(2) \times (t_{eff}/DT)$
<i>Unsatisfactory cheese, <math>P_{uc}</math></i>			
Threshold	Constant	Log cfu g <sup>-1</sup>	$10^6$ , $10^5$ , $10^7$ , or $10^8$
	Variable	Log cfu g <sup>-1</sup>	Triang(5,6,8) or Uniform(5,8)

<sup>a</sup> Based on expert opinion.

log–log transformed to obtain a simple, linear, relationship between doubling time and temperature that could be used in the simulation model (Table 2). The doubling time was then used in combination with a simple growth model to predict the log numbers at the selected storage conditions (Table 2). The lower pH-value may be representative for cheeses produced using a starter culture, whereas the higher was the average pH when 48 unripened cheeses of different types were analysed between 1988 and 1994 (pH=4.9 to 7.3, Björn Walldén, pers. comm.). It was assumed that there was a lag-phase before growth was initiated and a relationship between lag-phase and temperature was calculated in the same way as for doubling time (Table 2).

### 3.3.3. Consumption

The absence of a dose–response model made it pointless to consider consumption in the exposure assessment. In order to evaluate risk at the consumer level, preparation (including cross-contamination) and consumption steps need to be incorporated into the exposure assessment. Since data on consumer behaviour is scarce simplifying assumptions often have to be made. This constitutes a serious data gap and consequently a major source of uncertainty.

### 3.4. Risk characterisation

Since consumption was not considered and a dose–response model was lacking the present study was not a complete risk assessment. Instead the level of bacteria per gram was used as a proxy for the potential SET concentration and hence, for the potential risk to cause illness. The assessment endpoint selected for evaluation purposes was the probability that an unripened cheese made from raw milk contained at least 6 log cfu *S. aureus* g<sup>-1</sup> at the time of consumption. This endpoint was termed  $P_{uc}$ , the probability of an unsatisfactory cheese. The relation of this endpoint to public health was not defined and it should be emphasised that in order to fully assess the impact of a hazard both to an individual and to society, a dose–response model and a full exposure assessment including consumption is necessary.

A spreadsheet model was developed in Excel, and simulated using the @Risk software version 4.0 (Palisade, NY, USA). A simulation consisted of

10,000 iterations using Latin Hypercube sampling. The model is described schematically in Fig. 1. In developing the risk assessment, several assumptions were made and the influence of these assumptions on the magnitude of  $P_{uc}$  were also investigated (Fig. 1). To investigate the influence of the arbitrarily selected and constant threshold value we made a set of simulations using a constant threshold of 5, 7 and 8 log cfu g<sup>-1</sup>, respectively, or a variable threshold with distribution Triangular(5,6,8) and Uniform(5,8) log cfu g<sup>-1</sup>, respectively (Table 2). In addition, the influence of a lower proportion, 40%, of enterotoxigenic strains was also tested. This proportion was found in Norwegian cheeses made from raw milk (Kruse, 1999). To investigate the influence of a temperature limit for toxin production the model was calculated under the condition that the storage temperature must be above 10 °C for toxin production to occur. To investigate the influence of the minimum level of *S. aureus* in low level cheese, three simulations were carried out with the same random generator seed but using -2, -1, and 0 log cfu g<sup>-1</sup>, respectively, as the minimum value in the uniform distribution. The upper parameter was kept constant at 2 log cfu g<sup>-1</sup>.

The uncertainty in the estimation of the prevalence of *S. aureus* in cheese based on the survey can be described by a beta-function; Beta( $n-s+1$ ,  $s+1$ ), where  $n$  is the total number of samples and  $s$  is the number of positive samples (Vose, 2000). This beta-function describes the probability distribution of true prevalences, which could have yielded 11 positive samples if a total of 37 samples were analysed. To demonstrate the influence of this uncertainty on the simulated levels of *S. aureus* and  $P_{uc}$ , variability and uncertainty was separated in a second-order model. The model calculated variability, and the effect of uncertainty was investigated by simulating the model 10 times. Prior to each simulation, a value for the prevalence was sampled from the probability distribution described by Beta(27, 12).

#### 3.4.1. Results of risk characterisation

##### 3.4.1.1. Simulated levels of *S. aureus* in cheese.

Based on the mean or the median of the model parameters, the simulated level of *S. aureus* was always below the threshold, while it was always above the

threshold in the worst case-scenario (data not shown). Thus, point estimates was not useful in this study and a probabilistic approach was used in the following.

The simulated level of bacteria in low pH cheese was generally lower than in high pH cheese, due to the slower growth in a more acid environment (Fig. 2a). For instance, the probability that a cheese contains less than 1000 cfu *S. aureus* g<sup>-1</sup> at the time of

consumption was 0.67 (high pH) and 0.74 (low pH), respectively (Fig. 2b).

The final level of bacteria at the time of consumption depends on the interplay between the model parameters and it does not take exceedingly large values for unsatisfactory levels of bacteria to result (Table 3). In high pH cheese, the lowest storage temperature resulting in an unsatisfactory cheese

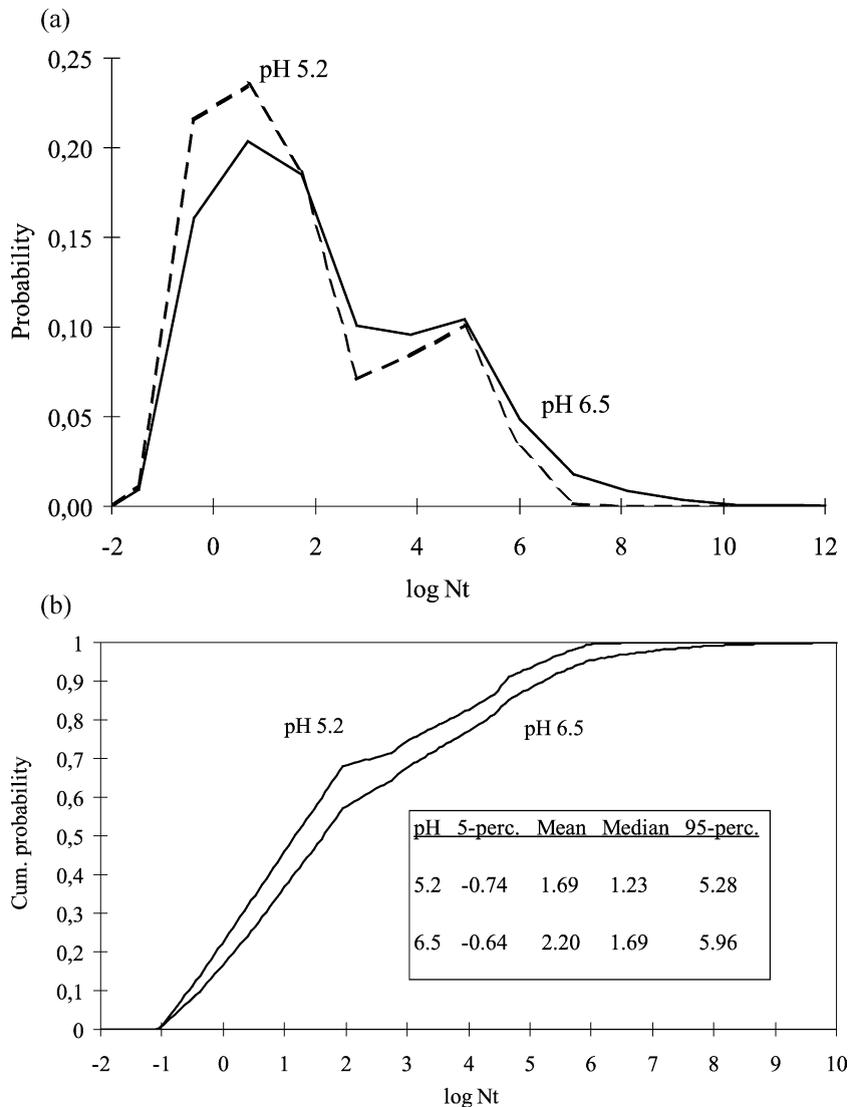


Fig. 2. Simulated levels of *S. aureus*, log  $N_t$ , at the time of consumption. A comparison of the levels in high and low pH cheese, respectively, presented in (a) as a probability distribution plot, and in (b) as a cumulative probability distribution plot. The table insert shows the 5 percentile, 95 percentile, the mean, and median of the simulated level (log cfu g<sup>-1</sup>).

Table 3

Some parameter combinations that resulted in simulated levels above the threshold selected for an unsatisfactory cheese, 6 log cfu g<sup>-1</sup>

Final level (log cfu g <sup>-1</sup> )	Initial level (log cfu g <sup>-1</sup> )	Storage temperature (°C)	Storage time (h)
<i>pH=5.2</i>			
6.0 min	5.2	11.4	89
6.2	3.9 min	12.3	125
6.1	6.0	9.0 min	125
6.4	5.7	12.1	68 min
<i>pH=6.5</i>			
6.0 min	4.8	9.3	93
6.6	0.35 min	11.8	145
6.3	5.7	7.5 min	136
6.1	5.7	10.8	39 min

Min indicates the lowest value for each parameter occurring in these iterations.

was 7.5 °C, the shortest storage time 39 h, and the lowest initial level of bacteria was 2 cfu g<sup>-1</sup>, i.e. below the detection limit of the analytic method used. The corresponding values in low pH cheese were 9.0 °C, 68 h, and 7900 cfu g<sup>-1</sup> (10<sup>3.9</sup>, Table 3). These numbers are for illustrative purposes and should not be taken as limits for cheeses of satisfactory quality.

A scenario analysis indicated that in both types of cheese the initial level of *S. aureus*, the storage temperature and time all contributed significantly to a *S. aureus* level above 6 log cfu g<sup>-1</sup>. At both pH values, the initial level of *S. aureus* were the most significant, followed by the storage temperature. In iterations yielding levels above this threshold, the median initial level in high pH was 4.6 log cfu g<sup>-1</sup>, the median storage temperature was 10.4 °C, and the median storage time was 111 h. In low pH cheese, the corresponding values were 5.4 log cfu g<sup>-1</sup>, 11.1 °C, and 114 h.

*3.4.1.2. Probability of an unsatisfactory cheese at the time of consumption depending on model assumptions.* Of all assumptions tested, the magnitude of the threshold had the greatest effect on the probability of an unsatisfactory cheese,  $P_{uc}$ . Using the 6 log cfu g<sup>-1</sup> threshold,  $P_{uc}$  was a factor of 8 lower in high pH cheese (0.048) than in low pH cheese (0.006). Treating the threshold as a variable instead of a constant (6 log cfu g<sup>-1</sup>) had practically no effect (high pH) or increased  $P_{uc}$  by less than a factor of 3 (low pH, Fig. 4). A change in threshold from 6 to 5 log cfu g<sup>-1</sup> increased  $P_{uc}$  by a factor of 13 for low pH cheese and a factor of 2.6 for high pH cheese (Fig. 4). Considering

all thresholds, the largest impact on  $P_{uc}$ , expressed as the ratio between the largest and the smallest estimate of  $P_{uc}$  was less than a factor of 36, except in one case; low pH cheese and a threshold of 5 log cfu g<sup>-1</sup> (Fig. 4). In comparison, the difference between cheeses as a function of pH was less than a factor of 30 (Fig. 4). Thus, the uncertainty introduced by the different assumptions was of the same order of magnitude (high pH) or 5 times larger (low pH), as the estimated variability of  $P_{uc}$  between the pH values examined.

Using 40% as the proportion of enterotoxigenic strains reduced  $P_{uc}$  by a factor of 2.5, while a 10 °C limit for toxin production reduced  $P_{uc}$  from 0.05 to 0.03 (high pH) and 0.006 to 0.005 (low pH). The relatively modest influence of a temperature limit on the magnitude of  $P_{uc}$  indicated that it was the high storage temperatures that had the largest influence on  $P_{uc}$  already in the first simulation. Although the minimum, the median, and the mean simulated level of *S. aureus* increased with an increasing minimum level of the uniform distribution, the influence on  $P_{uc}$  was negligible. For instance, in high pH cheese  $P_{uc}$  was 0.046, 0.047 and 0.051 for a minimum level of 1 cfu per 100 g, 1 cfu per 10 g, or 1 cfu per g, respectively.

*3.4.1.3. The effect of uncertainty in the prevalence estimate on the simulated level of S. aureus in cheese.* The effect of uncertainty of the prevalence estimate on the simulated level of *S. aureus* is illustrated by the difference between curves in Fig. 3. Based on 10 simulations, the mean (standard deviation) of  $P_{uc}$  at the time of consumption was 0.045 (0.009) and 0.006 (0.002) for a high and low pH

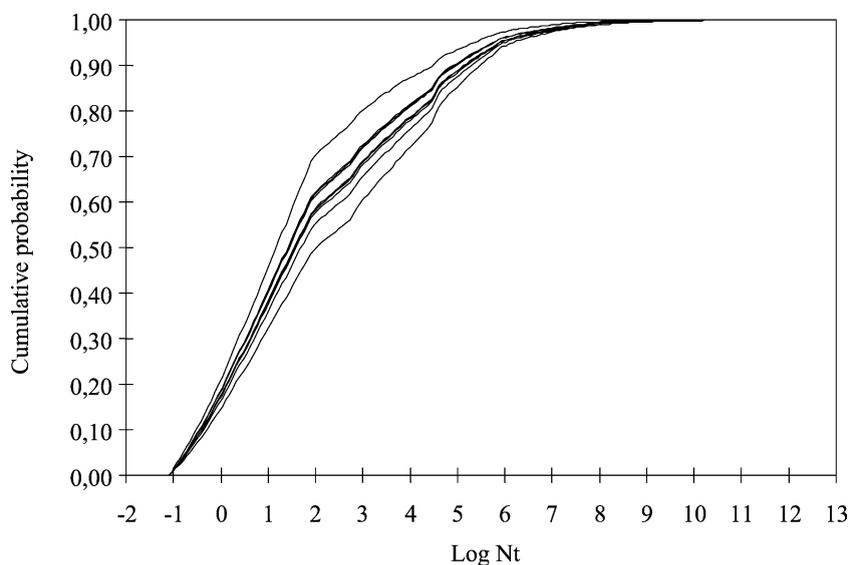


Fig. 3. The effect of uncertainty in the prevalence estimate on the simulated level of *S. aureus* at the time of consumption in high pH cheese. The results of 10 simulations of the second-order model, which separated variability and uncertainty, are shown as cumulative probability plots. The separate curves indicate the true variability within a simulation and the difference between curves is the effect of uncertainty in the estimated prevalence. Thus, in this assessment, the effect of true variability on the simulated levels dominated over the effect of uncertainty of the estimated prevalence.

cheese, respectively, and the ranges was 0.024 to 0.060 (high pH), and 0.002 to 0.008 (low pH). This contribution was modest in comparison with the true variability in the system reflected by the range of bacterial levels within a simulation (Fig. 3).

#### 3.4.2. Discussion

To evaluate the potential risk associated with unripened cheese made from raw milk, predictive microbiology and survey data was combined with probabilistic modelling to simulate the level of *S. aureus* at the time of consumption. Due to limited data and absence of dose–response models, a complete risk assessment and estimation of risk was not possible. Despite these limitations this type of quantitative assessment was still worthwhile since it was possible to gain insights and to evaluate several factors that influence the potential risk, e.g. pH, the initial level of *S. aureus*, storage conditions. In addition, any simulation study build on existing knowledge about the system, and the identification of gaps in the scientific knowledge is also a useful outcome of a modelling exercise (Jordan et al., 1999). However, before using a model for predictive purposes an

appreciation of its limitations is necessary since the results are valid only to the extent that data reflect true conditions and model assumptions are valid.

The result of the assessment indicated that a large fraction of the cheeses could contain unsatisfactory levels of *S. aureus* at the time of consumption. Staphylococcal food poisoning due to cheese has not been reported to any great extent to the authorities. Despite the fact that there is a substantial underreporting (Lindqvist et al., 2001), this may suggest that the potential risk as defined here overestimates the real risk. The environmental conditions during which *S. aureus* are able to form toxin are more restricted than those at which they can grow. This and our choice of threshold, which was at the lower end of those levels that have been reported in connection with outbreaks (Gockler et al., 1988; Otero et al., 1988), were in favour of such an interpretation. Most likely there is a large variation of the actual threshold depending on the bacterial strain, the type of food, and the individual consuming the food. However, describing the threshold as a variable instead of a constant (6 log cfu g<sup>-1</sup>), had a fairly limited effect on  $P_{uc}$  (Fig. 4). Similarly, the maximum relative impact of the other

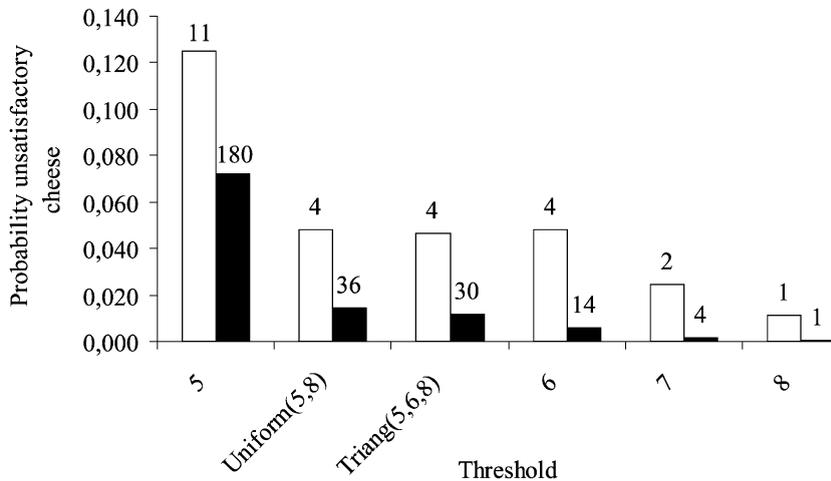


Fig. 4. The estimated probabilities for an unsatisfactory cheese,  $P_{uc}$ , depending on assumptions on the magnitude and distribution of the threshold level. The threshold level was assumed to be either constant at 5, 6, 7 or 8 log cfu  $g^{-1}$ , respectively, or distributed as Uniform(5,8) or Triang(5,5,8).  $P_{uc}$  was generally higher in high pH cheese (white bars) than in low pH cheese (black bars). The number above the bar is the ratio between  $P_{uc}$  and the lowest  $P_{uc}$  (threshold of 8 log cfu  $g^{-1}$ ) within high and low pH cheese, respectively.

model assumptions on  $P_{uc}$  was also fairly limited, except in the case of a constant threshold of 5 log cfu  $g^{-1}$  for a low pH cheese (Fig. 4). To simplify the assessment, only two pH values were used in the study. At both these pH values *S. aureus* can grow but we have limited information on the relative distribution of pH in these types of cheeses. The lower pH, 5.2, may be representative for growth in cheeses where starter culture have been added. It is also possible that the predictive growth model overestimated growth, since it was based on experiments in broth culture without a competing background flora. The growth of *S. aureus* in dairy products is often hampered by the presence of competitors (Halpin-Dohnalek and Marth, 1989).

The second-order model attempted to illustrate a way to resolve the variability and uncertainty of one of the model parameters. This analysis enabled us to estimate the impact of the uncertainty on the outcome of the model and indicated that variability not the uncertainty of the prevalence estimate dominated the total variability of the output, i.e. the level of *S. aureus*. Thus, efforts to better describe the variability of the initial levels of *S. aureus* should be more worthwhile than studies of the prevalence. This is not unexpected since initial levels range several orders of magnitude. How well the samples in the survey

represent these types of cheeses was not taken into account in this analysis. It should be pointed out that there is uncertainty associated also with the parameters describing variability, e.g. initial level of bacteria, storage temperature and time, and growth parameters, but this uncertainty was not addressed. Nauta (2000) introduced a parameter  $\alpha_x$  representing the fraction of the total variance attributable to uncertainty for parameter  $x$ , and  $1-\alpha_x$  representing the fraction due to variability, to accommodate a situation where an input distribution reflects both variability and uncertainty. By selecting arbitrary values for the  $\alpha$  parameter for two of the model parameters the impact on the output results was illustrated (Nauta, 2000). The discussion about uncertainty and variability is analogous to the discussion in diagnostic epidemiology of the validity, i.e. the lack of bias, versus the precision, i.e. the observed variability, of a test (Martin, 1988; Gardener and Greiner, 1999). It has been the wisdom that one should always give priority to the valid estimate, not necessarily the estimate with the best precision.

The importance of assumptions concerning the level of bacteria in samples in which the hazard was not detected was emphasised by the finding that storage of a “negative” cheese (high pH, 2 cfu  $g^{-1}$ ) could lead to levels above the threshold (Table 3). Our assumption about the distribution of bacteria in the

negative samples contributed to the uncertainty of the assessment, but the result illustrated the importance of interpreting negative analytical results in relation to the method used in the analysis. For example, in the US-FDA/FSIS (2000), risk assessment on *Listeria monocytogenes*, samples negative in qualitative analyses were assumed to contain less than  $0.04 \text{ cfu g}^{-1}$ , i.e. 1 in 25 g.

An extensive exposure assessment may require the specification of over 100 model parameters. Only a few of these inputs drive the assessment in terms of having a substantial impact on the magnitude or the ranges of predicted risks. The use of probabilistic techniques may be restricted to pathways and processes that have major effects on the exposure or for understanding the exposure. This can save resources in the analysis by simplification of the model without compromising the scientific integrity or usefulness to a risk manager. Zwietering and van Gerwen (2000) presented a technique employing sensitivity analysis and going from point-estimates over a worst-case scenario to a stochastic analysis to identify just those steps in the assessment most contributing to the variability of the output. In the present study, the uncertainty of the growth process was not addressed with probabilistic techniques despite its importance for the assessment. A limitation of predictive growth models is that most of them were not developed for use in probabilistic exposure assessments, and uncertainty or biological variability is only scarcely incorporated in predictive microbiology models (Nauta and Dufrenne, 1999). Consequently, for most of them, information on the uncertainty and variability associated with the model parameters are lacking, or the distinction between them is not made (Nauta, 2001).

The finding that initial levels below detection could result in an unsatisfactory cheese illustrated the importance of growth for the safety of these products. Based on the present assessment, some inferences with relevance to risk management can be made. In high pH cheese, even low regulatory limits, for instance  $100 \text{ cfu g}^{-1}$ , would not necessarily be effective (Table 3), whereas in low pH cheese higher levels than that may be safe (Table 3). Thus, managing the cheese making process to obtain a pH around 5, for instance by adding buttermilk as a starter, would limit growth and increase the safety of these products substantially. A low pH also reduces the ability of

enterotoxigenic strains to produce SET toxin (Halpin-Dohnalek and Marth, 1989). Management options directed at reducing the variability and initial level of *S. aureus* in cheeses may also be effective and need to be addressed in improved versions of the model.

Data gaps that were identified included the occurrence, but especially the variation in the level of *S. aureus* and SET in these products, domestic storage temperatures and times, and the occurrence of staphylococcal food poisoning due to these cheeses. Another knowledge gap concerns our limited understanding of how the dose–response relationships for toxin producing microorganisms such as *S. aureus* are best described. Due to this gap the exposure assessment was limited to the level of contamination in the food product independent of consumption patterns, and the consumption and preparation steps were not explicitly addressed. A crucial assumption in our assessment was the presence of a lag phase before growth was initiated. In the absence of a lag phase, the simulated levels of *S. aureus* and the implications of this study would be much different. To address some of the identified limitations, a survey of food storage temperatures is now underway in Sweden, as is a study aiming at investigating the growth and toxin production in these types of cheeses. Finally, other hazards in addition to *S. aureus* may be present in raw milk and may constitute potential health problems, e.g. *Escherichia coli* O157:H7, *L. monocytogenes*, *Campylobacter*, but these were not addressed in this study.

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