ELSEVIER

International Journal of Food Microbiology 73 (2002) 297–304

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Modelling bacterial growth in quantitative microbiological risk assessment: is it possible?

Maarten J. Nauta *

Microbiological Laboratory for Health Protection, National Institute of Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands

Received 16 May 2001; accepted 9 August 2001

Abstract

Quantitative microbiological risk assessment (QMRA), predictive modelling and HACCP may be used as tools to increase food safety and can be integrated fruitfully for many purposes. However, when QMRA is applied for public health issues like the evaluation of the status of public health, existing predictive models may not be suited to model bacterial growth. In this context, precise quantification of risks is more important than in the context of food manufacturing alone. In this paper, the modular process risk model (MPRM) is briefly introduced as a QMRA modelling framework. This framework can be used to model the transmission of pathogens through any food pathway, by assigning one of six basic processes (modules) to each of the processing steps. Bacterial growth is one of these basic processes. For QMRA, models of bacterial growth need to be expressed in terms of probability, for example to predict the probability that a critical concentration is reached within a certain amount of time. In contrast, available predictive models are developed and validated to produce point estimates of population sizes and therefore do not fit with this requirement. Recent experience from a European risk assessment project is discussed to illustrate some of the problems that may arise when predictive growth models are used in QMRA. It is suggested that a new type of predictive models needs to be developed that incorporates modelling of variability and uncertainty in growth.

Keywords: Quantitative microbiological risk assessment; Growth models; Variability; Uncertainty

1. Introduction

Quantitative microbiological risk assessment (QMRA), predictive modelling and HACCP have gained increased attention in food microbiology in recent years. They offer structures and tools to enhance food safety by evaluating the safety of (new) food products and predicting the effects of intervention measures in food production processes. Although the three are integrated (Buchanan and Whiting, 1996; Notermans and Mead, 1996; Elliott, 1996; Walls and Scott, 1997; Buchanan and Whiting, 1998; Serra et al., 1999), there are some important differences. Whereas HACCP is typically linked to industrial processes, QMRA is more often used for public health purposes, for example when 'farm to table' models are constructed. Both in the HACCP system and in QMRA studies, potential bacterial growth can be incorporated by applying predictive food microbiology models. Predictive models can quantify the increase or de-

* Tel.: +31-30-2742805; fax: +31-30-2744434.
E-mail address: Maarten.Nauta@rivm.nl (M.J. Nauta).

0168-1605/02/$ - see front matter
PII: S0168-1605(01)00664-X

crease of bacterial population sizes. However, these models have not been specifically developed for the purpose of QMRA. As with mathematical models in general, one should be careful in applying them for other purposes than those for which they are made. Research questions that led to the modelling may have induced simplifying assumptions that are not valid in other circumstances.

Therefore, the applicability of predictive microbiology models in quantitative microbiological risk assessment is discussed in this paper. It focuses on bacterial growth as a typical QMRA process. First, general aspects of QMRA are discussed. The modular process risk model (MPRM) framework, which is currently being developed by the author, is introduced and the concepts of risk and probability are briefly reviewed. Second, an overview is given of predictive growth modelling, and the discrepancy between the output of growth models and the needs of QMRA is indicated. Third, the problems are illustrated by some experiences that we had in a recent case study of *Bacillus cereus* in a vegetable product. In this case study, some additional problems could be identified. Finally, possibilities for increasing the usefulness of predictive growth models for QMRA are discussed.

2. Quantitative microbiological risk assessment (QMRA)

2.1. The modular process risk model (MPRM) framework

Several QMRA studies, on for example *Salmonella enteritidis* in eggs, *Escherichia coli* O157:H7 in beef, *Listeria monocytogenes* in cheese and *Salmonella* spp. and *Campylobacter* spp. in poultry, are completed or in progress (e.g. Whiting and Buchanan, 1997; Cassin et al., 1998; Bemrah et al., 1998). In these studies, the transmission of the hazard involved is modelled through the food pathway, a chain of processes from a source (e.g. the farm) to the moment of consumption. This transmission model follows (probability distributions of) the prevalence and the concentration of the hazard along the consecutive processes of the food pathway. The resulting risk model may not only be used to assess the current risk of the hazard/product/process combination, but also to predict the effects of interventions proposed to mitigate the risk.

Several approaches have been suggested for such ‘farm to table’ QMRA models (McNab, 1998; Cassin et al., 1998; Marks et al.,1998). As a general framework, we propose the use of ‘modular process risk models’ (MPRMs) (Nauta, 2001), a variant of the Process Risk Model introduced by Cassin et al. (1998). In short, the idea behind MPRMs is that in any food pathway, all processing steps can be identified as one of six basic processes (modules): either one of two microbial processes, growth and inactivation, or one of four product handling processes, mixing, partitioning, removal and cross contamination. In principle, once the modelling techniques for all these basic processes are established, every food pathway can be modelled. The food pathway is split up in a series of processing steps and one basic process is assigned to each of these steps. If properly defined, the input and output of all the models for the basic processes can be linked, and any food pathway can be modelled. An important characteristic of the MPRM is that it is process-driven and not data-driven, that is that the models should be mechanistic as much as possible. The choice of the model should be based on the process, not on the availability of data.

Table 1 gives an overview of the effects of the six basic processes on the prevalence (the fraction of contaminated ‘units’), the concentration (that is the number of microorganisms per ‘unit’) and the unit size. The definition of the ‘unit’ is crucial here. It is a physically separated quantity of product in the process, like for example a carcass, a package of ground beef, a milk tank or a bottle of milk. If units are mixed or partitioned, the unit has to be redefined, and the distribution of microorganisms over the units is altered. As an example, consider contaminated milk in a large tank that is distributed over a large number of milk bottles: the unit size decreases, the prevalence may decrease if by chance a bottle ends up uncontaminated, but the total number of cells remains equal.

From the basic processes mentioned above, the process that is most typical for microbial risk assessment is microbial growth. Growth is a complicated, uncertain and variable process. As can be read from Table 1, it is the only basic process (with cross

Table 1
Basic processes of the MPRM and their qualitative effect on the prevalence (P), the number of organism in all units (N_{tot}) and the unit size

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

=: no effect.
+: an increase.
−: a decrease.

contamination) which will always give an increase of risk. Due to growth, products that are contaminated at a low level (possibly below the detection limit) and therefore are considered safe at a certain moment of time may become unsafe at some later time. This is an important issue in for example the context of 'Food Safety Objectives'.

This paper concentrates on the basic process of microbial growth. The aim is to find a modelling technique for microbial growth that is suited to implement in a MPRM for QMRA. As growth modelling is widely applied in predictive microbiology, we regard predictive microbiology models in the light of the needs for QMRA.

2.2. The aims of QMRA

Although 'food safety' is a common goal, food industry and public health authorities have different aims when applying QMRA, HACCP or predictive modelling. HACCP is particularly relevant for food manufacturers and food catering, which characteristically deal with well-defined and controlled production and/or preparation processes. Both quantitative risk assessment and predictive modelling can be used in the HACCP system (Notermans and Mead, 1996; Elliott, 1996; Buchanan and Whiting, 1998; Serra et al., 1999). In a HACCP context, the purpose of these two tools is to assess whether a food product will be safe at the moment of consumption. Risks are eliminated or reduced as much as possible. For public health authorities, however, risk assessment may serve as a means to quantify the risks attributable to certain food products. In that case, the purpose of quantitative risk assessment is not so much the production of safe food, but an evaluation of the health status of the population. Quantification of risks is therefore more important when QMRA is applied for public health purposes. By applying QMRA and using integrated public health measures, risks of a different nature can be compared. For example, Havelaar et al. (2000) used this approach to compare the risks of infection by *Cryptosporidium parvum* in drinking water, to the risk of renal cell cancer as a consequence of decontamination of the water. So, although both industry and public health workers may use QMRA, HACCP and predictive modelling techniques, they often have different aims. This may have severe consequences for the modelling.

Important concepts in risk assessment are 'risk', 'probability' and 'probability distribution'. Several definitions of risk are going around. According to Notermans et al. (1996), risk is simply 'the probability that an adverse effect will occur', whereas in the context of QMRA, it is more precisely defined as 'a function of probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food, (CODEX Alimentarius Commission, 1998) or 'the product of the likelihood of the occurrence and the magnitude of the consequences of exposure to a pathogen on human health' (ILSI, 2000). In all these definitions of risk, the term 'probability' (or 'likelihood') appears. This probability is a quantitative measure, a number between zero and one expressing the odds on an event. If a parameter or a variable can have different values, and we know how probable

these values are, these probabilities can be represented by a 'probability distribution'. In QMRA, such probability distributions play an important role (Vose, 1998).

An important aspect of probability distributions is that they can represent either uncertainty or variability (Hattis and Burmaster, 1994; Murphy, 1998; Anderson and Hattis, 1999). 'Uncertainty' represents the lack of perfect knowledge of the parameter value, which may be reduced by further measurements. 'Variability', on the other hand, represents a true heterogeneity of the population that is a consequence of the physical system and irreducible by additional measurements. In the context of microbial growth modelling, uncertainty may for example result from imprecise measurements or lack of knowledge of the effects of conditions that are not included in the model. Variability may result from variability in temperature, strain differences or other sources of biological variability. As recently illustrated, the separation of uncertainty and variability, by using second order Monte Carlo models, may be important in QMRA (Nauta, 2000b).

All this implies that 'probability' is a crucial concept in risk assessment. For safe food production, evaluation of risk may be less important, as it 'just' means to keep risk low. For public health objectives, risk has to be evaluated quantitatively, both for reasons of comparison with other health risks and for the evaluation of proposed risk mitigation strategies (Nauta et al., 2000). A risk assessment therefore has to incorporate probabilities throughout the analysis. This may demand a special type of predictive modelling (Nauta, 2000a).

3. Growth modelling

Bacterial growth can basically be regarded as an increase in population size. If the aim is to assess bacterial growth in, e.g. a MPRM processing step, we essentially want to assess the population size (N_{out}) of a pathogen at the end of a processing step, given an initial population size (N_{in}) and the process itself. This is illustrated schematically in Fig. 1.

Fig. 1. The aim of growth modelling in risk assessment is essentially the assessment of the population size N_{out} at the end of a processing step, given the initial population size N_{in} and the process.

In general, any predictive growth model has the structure

$$\log(N_{out}) = \log(N_{in}) + f(\cdot) \quad (1)$$

with N_{in} the number of cells at the beginning of the process, N_{out} the number of cells at the end of the process and $f(\cdot)$ an (increasing, positive) growth function. When a number of consecutive processing steps is considered, $f(\cdot)$ of a single step is equal to the 'step characteristic' of that step as defined by Van Gerwen and Zwietering (1998). The growth function $f(\cdot)$ can have many shapes, which are widely discussed in predictive modelling literature (e.g. McMeekin et al., 1993; Whiting, 1995; Van Gerwen and Zwietering, 1998). For example, for exponential growth $f(t) = \mu t$ (with t is time and μ is the specific growth rate), for the lag exponential model $f(t) = \mu(t - \lambda)$ (with λ lag time duration), and when using the Gompertz equation $f(t) = a \exp[-\exp(b - ct)]$, with parameters a, b and c. Note that all these are 'primary growth models', which are a function of time.

In general, the selection of the 'best' growth model depends on the modelling purpose, process knowledge and data availability. If, for example, a change in the temperature regime is considered, it will be necessary to use a 'secondary growth model', which incorporates 'temperature' as a parameter. In that case, the growth function $f(\cdot)$ will be a function of both time and temperature. Which specific secondary growth model to select for this purpose, will depend on previous experience and the availability of data.

As stated in the previous chapter, it is important to realize that a quantitative microbiological growth prediction has usually different demands than a 'traditional' predictive food microbiology growth model prediction. The latter is developed to come to a growth curve prediction, that is a series of point estimates of population size for a time series. However, as illustrated in Fig. 2, in a QMRA model point estimates are not sufficient. Moreover, when the processing time is fixed, time is not a variable and it may be irrelevant to predict the dynamics in time. As

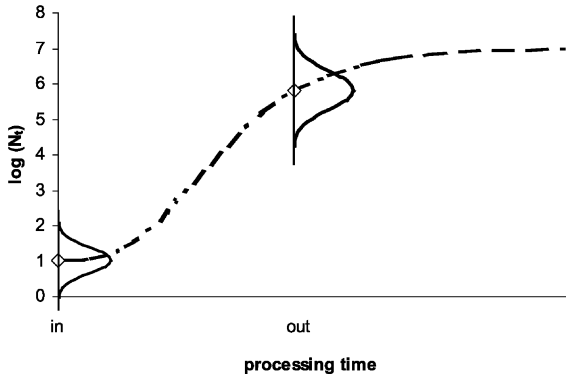

Fig. 2. Growth is the increase in population size, given as $\log(N_t)$, as a function of time. Predictive microbiology models typically predict a growth curve, as given by the dashed line. In these models, growth is considered as a function of time and the model yields a point estimate at any point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

explained, risk assessment is about probabilities of events, so here we are interested in the probability distribution of the population size, that is the probability that a microbial population will reach a certain level, or the probability that a certain level is reached within a certain amount of time (Whiting, 1997; Soboleva et al., 2000). In the terminology used here, this means we are not so much interested in $f(\cdot)$ as a function of time, but mainly in the probability distribution(s) of $f(\cdot)$ (the step characteristic) at the end of the processing step (Nauta, 2000a). With some exceptions, for example time-to-growth models (Whiting, 1995), predictive models do not predict probabilities, but changes in concentrations. Therefore, they cannot be directly implemented in a QMRA.

An obvious solution to this problem may be to use a probability distribution for N_{in} and predict the probability distribution of N_{out}, e.g. by Monte Carlo modelling, using a selected predictive modelling equation. If probability distributions of model parameters (like μ or λ) are derived too, it is easy to implement it all together in one Monte Carlo model. This method will yield a nice looking probability distribution of N_{out}. However, one should be very careful here. Predictive models that incorporate the effect of growth conditions on the growth curve are usually not validated for predictions under uncertain and/or variable conditions as in the food pathway modelled. There is no reason to believe that the model predictions are still correct, if point estimates are replaced by probability distributions of the input parameters of the model (Nauta and Dufrenne, 1999).

Two major pitfalls can be identified here. The first is that probability distributions are mixed that should be separated, for example because one describes uncertainty and the other variability, or because one describes variability within a strain, and the other variability between strains (Nauta, 2000b). The second is that some sources of variability and uncertainty may be neglected. This pitfall lies in wait when experimental data are implemented in a QMRA that covers a broader range of strains and conditions than used in the experiments. Sources of variability and uncertainty are strain differences, within strain biological variability, model uncertainty, variability and uncertainty in processing conditions, variability and uncertainty in food composition, etc. It is very difficult to account for all this in a risk assessment model. The consequence of the pitfalls may not only be that a risk is assessed with a level of certainty that is heavily overestimated, but also an improper estimate of the risk (Nauta, 2000b).

4. Experience with a case study: additional problems

Recently, we had some experience with a case study that was part of an EU project, a QMRA on a sporeforming pathogen, *B. cereus*, in a vegetable product (Carlin et al., 2000; Nauta, 2001). In this risk assessment, the data allowed quantification of the variability of the initial contamination characteristics, and variability in time and temperature profiles along the food pathway. As some major sources of uncertainty could not be quantified, uncertainty was omitted from our analysis. As explained above, for the microbial process growth and inactivation the quantification of variability was not straightforward. Nevertheless, we intended to have an exercise in constructing a MPRM and had to end up with a risk estimate, so we decided to model growth and inacti-

vation as simple as possible within the MPRM framework.

As a solution to the problems identified, we chose to use a lag exponential growth model, fitted to growth data of five different *B. cereus* strains, acquired with the project. As time and temperature were variable along the food pathway modelled, we had to use a second order growth model. For this, we selected the square root model for the specific growth rate μ and an inverse square root model for the lag time λ (Ratkowsky et al., 1982; Wijtzes et al., 1995), all on the basis of practicability and simplicity.

$$\mu = b(T - T_{\min})^2 \tag{2}$$

with b a parameter, T the temperature and $T_{\min}$ the minimum growth temperature, and

$$\lambda = c(T - T_{\min})^{-2} \tag{3}$$

with c a parameter, so

$$f(T,t) = b((T - T_{\min})^2 t - c) \tag{4}$$

and

$$\ln(N_t) = \ln(N_0) + b((T - T_{\min})^2 t - c). \tag{5}$$

For each strain, the standard deviation in the estimates of the model parameters, as derived from the microbiological data using regression analysis, was used to assess the variability in growth per Monte Carlo simulation run. This is a simple, non-validated, method, which neglects many aspects of growth modelling for QMRA purposes, as discussed in this paper. Among others, this method uses the uncertainty and variability in the model parameters in Eq. (5), to predict the variability in growth only.

The QMRA in the case study did not yield a precise risk estimate, and had limited value for public health purposes. However, it could identify gaps in knowledge, was an instructive exercise in using the MPRM methodology and even identified some promising risk mitigation strategies (Nauta, 2001).

The attempts to use predictive modelling for QMRA taught us some interesting lessons. Not only were the identified problems in growth modelling confirmed, additionally it was found that: (1) the availability of applicable predictive models for lag time under temperature regimes with changing temperatures is indispensable for QMRA modelling, especially when consumer transport, storage and preparation is part of the food pathway modelled; and (2) modelling of growth and inactivation of sporeformers requires quantitative predictive models for spore germination and sporulation. As inactivation models usually describe the fate of spores, and growth models describe the fate of 'colony forming units', one should be extremely careful with linking growth and inactivation models without considering the state of the cells.

5. Possibilities of modelling growth in QMRA

As outlined in the chapters above, it is not straightforward to apply the available predictive microbiology growth models in QMRA studies. This holds especially for QMRA studies that aim to evaluate the status of public health with regards to a specific hazard and/or food product. QMRA studies assess probabilities and therefore need to use stochastic models, preferably second order Monte Carlo models. Predictive growth models are generally developed and validated as models that give point estimates, not as stochastic models.

Of course, predictive models are highly valuable for many food safety purposes, like the development of HACCP plans and evaluation of the safety of steps in the food production process. Predictive models may be considered as 'worst case' predictions (see, e.g. Zwietering et al., 1996). As such, they are useful to identify non-safe processing steps. They are however not suited to quantify risks.

Several authors have recognised the importance of incorporating variability in predictive growth modelling. In a paper on choosing probability distributions for modelling variability in growth, Ratkowsky et al. (1996) find that the Gamma distribution is a suitable stochastic assumption when modelling generation times. Soboleva et al. (2000) argue that it is not appropriate to a priori select a probability distribution for the population size. They develop a method to describe the population size in time by incorporating random errors in the parameters of the differential equations describing growth. Marks and Coleman (unpublished) present a way to model the natural

variability in growth and inactivation by the application of birth and death process dynamics. All these approaches show that the probability distribution of the increase in population size (that is $f(\cdot)$ as in Eq. (1)) and thus the population size after growth will be skewed, with a long tail for large population sizes. Of course, this is particularly relevant in the context of risks. However, the studies on stochasticity in growth, have not yet yielded predictive models that are generally applicable in QMRA. Among others because data fitting has not been put in the light of estimating probability distribution parameters or separation of uncertainty and variability. It may for example be that the variability is skewed, but the uncertainty is not.

In risk assessment, risk estimates have to be provided with an indication of the attendant uncertainty (CODEX Alimentarius Commission, 1998). This implies that an uncertainty analysis should be performed, and that where possible all uncertainties in the risk assessment should be quantified. Incorporation of such an uncertainty analysis may not only give insight in the reliability of the risk estimate, it may also provide a tool to identify the most important gaps in knowledge along the food pathway and thus address where additional research is necessary. However, like in the case study mentioned above, quantification of uncertainty may be problematic. One can use statistics to get an uncertainty interval about some parameter estimates, but when data are difficult to interpret and validated models are not available, this 'statistic uncertainty' need not reflect the 'real uncertainty'. In that case, an evaluation of expert opinions or scenario analysis may be used to get insight in uncertainty (Nauta et al., 2000).

The title of this paper holds the question whether bacterial growth modelling for QMRA is possible. Based on the arguments given, the answer seems to be: not yet. The development of predictive growth models has yielded large data bases, various modelling techniques, and valuable insight in growth kinetics (Whiting, 1995; USDA, 1998; Van Gerwen and Zwietering, 1998). Especially mechanistic models based on growth kinetics (Van Impe et al., 1992; Baranyi and Roberts, 1994) would fit in the MPRM philosophy. Special models should be developed with QMRA in mind, with identification and quantification of specific sources of variability and uncertainty. Validation of these models may in part be possible on the basis of available data, but new experiments that are set up to specifically characterise variability and uncertainty will be necessary as well. If this is established, the power of both predictive modelling and QMRA will be enlarged. Therefore, it can be regarded as a major challenge for the future.

Acknowledgements

Part of the work described here was carried out as part of the EU project FAIR CT97-3159 (RASP), and many ideas are formed thanks to the stimulating discussion with participants within this project. The importance of modelling germination was pointed out to me by Dr. Gary Barker (Institute of Food Research, Norwich, UK).

References

Anderson, E.T., Hattis, D., 1999. Uncertainty and variability. Risk Analysis 19, 47–49.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology 23, 277–294.

Bemrah, N., Sanaa, M., Cassin, M.H., Griffiths, M.W., Cerf, O., 1998. Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk. Preventive Veterinary Medicine 37, 129–145.

Buchanan, R.L., Whiting, R.C., 1996. Risk assessment and predictive microbiology. Journal of Food Protection, 31–36.

Buchanan, R.L., Whiting, R.C., 1998. Risk assessment: a means for linking HACCP plans and public health. Journal of Food Protection 61, 1531–1534.

Carlin, F., Girardin, H., Peck, M.W., Stringer, S., Barker, G., Martinez, A., Fernandez, P., Waites, W.M., Movahedi, S., Van Leusden, F., Nauta, M.J., Moezelaar, R., Del Torre, M., Litman, S., 2000. A FAIR collaborative programme: research on factors allowing a risk assessment of spore-forming pathogenic bacteria in cooked chilled foods containing vegetables (FAIR CT97-3159). International Journal of Food Microbiology 60, 117–135.

Cassin, M.H., Lammerding, A.M., Todd, E.C.D., Ross, W., McColl, R.S., 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. International Journal of Food Microbiology 41, 21–44.

CODEX Alimentarius Commission, 1998. Proposed draft principles and guidelines for the conduct of microbial risk assessment. Codex Committe on Food Hygiene (CX/FH 98/2), Food and Agriculture Organization of the United Nations/World Health Organization, Rome, Italy.

Elliott, P.H., 1996. Predictive microbiology and HACCP. Journal of Food Protection, 48–53.

Hattis, D., Burmaster, D.E., 1994. Assessment of variability and uncertainty distributions for practical risk analyses. Risk Analysis 14, 713–730.

Havelaar, A.H., De Hollander, A.E., Teunis, P.F., Evers, E.G., Van Kranen, H.J., Versteegh, J.F., Van Koten, J.E., Slob, W., 2000. Balancing the risks and benefits of drinking water disinfection: disability adjusted life-years on the scale. Environmental Health Perspectives 108, 315–321.

ILSI, 2000. Revised Framework for Microbial Risk Assessment, An ILSI Risk Science Institute Workshop Report. ILSI Press, Washington.

Marks, H.M., Coleman, M.E., Lin, C.-T.J., Roberts, T., 1998. Topics in microbial risk assessment. Dynamic flow tree process. Risk Analysis 18, 309–328.

McMeekin, T.A., Olley, J.N., Ross, T., Ratkowsky, D.A., 1993. Predictive Microbiology: Theory and Application. Wiley, New York.

McNab, W.B., 1998. A general framework illustrating an approach to quantitative microbial food safety risk assessment. Journal of Food Protection, 1216–1228.

Murphy, B.L., 1998. Dealing with uncertainty in risk assessment. Human and Ecological Risk Assessment 4, 685–699.

Nauta, M.J., 2000. Modelling bacterial growth in quantitative microbiological risk assessment: is it possible? In: Van Impe, J.F.M., Bernaerts, K. (Eds.), Predictive Modelling in Foods—Conference Proceedings. KULeuven/BioTeC, Belgium (ISBN 90-804818-3-1), pp. 243–245.

Nauta, M.J., 2000b. Separation of uncertainty and variability in quantitative microbial risk assessment models. International Journal of Food Microbiology 57, 9–18.

Nauta, M.J., 2001. A modular process risk model structure for quantitative microbiological risk assessment and its application in an exposure assessment of *Bacillus cereus* in a REPFED. report nr. 149106 007 RIVM, Bilthoven, The Netherlands.

Nauta, M.J., Dufrenne, J.B., 1999. Variability in growth characteristics of different *E. coli* O157:H7 isolates and its implications for predictive microbiology. Quantitative Microbiology 1, 137–155.

Nauta, M.J., Van de Giessen, A.W., Henken, A.M., 2000. A model for evaluating intervention strategies to control salmonella in the poultry meat production chain. Epidemiology and Infection 124, 365–373.

Notermans, S., Mead, G.C., 1996. Incorporation of elements of quantitative risk analysis in the HACCP system. International Journal of Food Microbiology 30, 157–173.

Notermans, S., Mead, G.C., Jouve, J.L., 1996. Food products and consumer protection: a conceptual approach and a glossary of terms. International Journal of Food Microbiology 30, 175–185.

Ratkowsky, D.A., Olley, J., McMeekin, T.A., Ball, A., 1982. Relationship between temperature and growth rate of bacterial cultures. Journal of Bacteriology 149, 1–5.

Ratkowsky, D.A., Ross, T., Macario, N., Dommett, T.W., Kamperman, L., 1996. Choosing probability distributions for modelling generation time variability. Journal of Applied Bacteriology 80, 131–137.

Serra, J.A., Domenech, E., Escriche, I., Martorell, S., 1999. Risk assessment and critical control points from the production perspective. International Journal of Food Microbiology 46, 9–26.

Soboleva, T.K., Pleasants, A.B., le Roux, G., 2000. Predictive microbiology and food safety. International Journal of Food Microbiology 57, 183–192.

USDA, 1998. Pathogen Modeling Program, version 5.1. Microbial Food Safety Unit, USDA, ARS Eastern Regional Research Centre, http://www.arserrc.gov/mfs/pathogen.htm.

Van Gerwen, S.J., Zwietering, M.H., 1998. Growth and inactivation models to be used in quantitative risk assessments. Journal of Food Protection 61, 1541–1549.

Van Impe, J.F., Nicolai, B.M., Martens, T., De Baerdemaeker, J., Vandewalle, J., 1992. Dynamic mathematical model to predict microbial growth and inactivation during food processing. Applied and Environmental Microbiology 58, 2901–2909.

Vose, D.J., 1998. The application of quantitative risk assessment to microbial food safety. Journal of Food Protection 61, 640–648.

Walls, I., Scott, V.N., 1997. Use of predictive microbiology in microbial food safety risk assessment. International Journal of Food Microbiology 36, 97–102.

Whiting, R.C., 1995. Microbial modeling in foods. Critical Reviews in Food Science and Nutrition 35, 467–494.

Whiting, R.C., 1997. Microbial database building: what have we learned? Food Technology 51, 82–87.

Whiting, R.C., Buchanan, R.L., 1997. Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasteurized liquid eggs. International Journal of Food Microbiology 36, 111–125.

Wijtzes, T., de Wit, J.C., Huis in't Veld, J.H.J., van't Riet, K., Zwietering, M.H., 1995. Modelling bacterial growth of *Lactobacillus curvatus* as a function of acidity and temperature. Applied and Environmental Microbiology 61, 2533–2539.

Zwietering, M.H., De Wit, J.C., Notermans, S., 1996. Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. International Journal of Food Microbiology 30, 55–70.