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HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C1 - Follow-up and dissemination of scientific opinions

PRELIMINARY REPORT

Risk assessment of food borne bacterial pathogens:

Quantitative methodology relevant for human exposure assessment

NOTE:
The report has been submitted to the SSC on 21-22 February 2002 as a preliminary document. It is based on data published in scientific journals or available from ongoing research projects. Other relevant information may, however, be available from other sources that do not commonly report in scientific or technical press.
Scientists, industrial associations, research institutes, veterinary pathology laboratories, etc. are therefore invited to comment on the attached documents and, if appropriate, provide additional information. These contributions should be sent before 15 June 2002 to the secretariat of the SSC. The SSC will if appropriate integrate them in its final opinion.
The industry is invited to, as far as possible, co-ordinate its comments and channel them through existing associations. Individual comments are, however, also welcome.

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Summary: This report is giving an overview of the present use of stochastic models in quantitative assessment of human exposure to food borne bacterial pathogens. The second chapter defines exposure assessment in the context of the overall risk analysis and stresses that the pivotal step in the whole risk analysis process is the risk evaluation step, where one identifies hazards, develops risk profiles, sets priorities and allocates resources; commissions risk assessments (including exposure assessments) and evaluate their results.

In the context of this report the exposure assessment provides an estimate of how likely it is that an individual or a population will be exposed to a microbial hazard and what numbers of organisms are likely to be ingested. It is stressed that the exposure assessment shall be seen as an iterative and continuous process.

The third chapter addresses the model development. Quantitative risk assessment, in particular when using stochastic models, is a specialised task that requires skills in mathematics and statistics in addition to microbiological and technological knowledge. As a consequence, risk assessments are usually conducted in large, multidisciplinary projects. Building a comprehensive model may be resource intensive. The output of risk models is relatively complex, and in order to guide the risk assessment and interpret the results, risk managers need to understand the basic principles of modelling and concepts like uncertainty and variability. A general framework for doing quantitative microbiological risk assessment (QMRA), the Modular Process Risk Model (MPRM) was recently proposed. The proposal is that to each of the steps at the intermediary stages of a farm-to-fork chain at least one of six basic processes can be assigned, i.e. specifically, growth, inactivation, partitioning, mixing, removal and cross contamination.

The next chapter dealing with the modelling of the food chain describes the different steps in the food chain as primary production, processing and retails, handling in private households and finally consumption patterns.

In chapter 5 is given 5 examples of recent modelling exercises: A Shiga toxin producing *E.coli* O157 in steak tartare patties, *Bacillus cereus* in broccoli puree, Soft cheese made from raw milk, Pathways to be included in risk assessment of *Campylobacter* in chickens, Cold smoking of fish as a generic fish HACCP example.

The types of data possibly used in an exposure assessment comprise data on the food product, the food chain, the microorganism and the consumer. Chapter 6 concludes it is crucial that risk assessors carefully communicate their data needs to both risk managers and scientists involved in observational or experimental studies, and that the latter promote incorporation of the necessary data collection efforts within current budgets.

The separation of variability and uncertainty in QMRA models has up to now rarely been made. Neglecting the difference between them may lead to improper risk estimates. If the distinction is not clear to the analyst, a variability distribution may incorrectly be used as if it was an uncertainty distribution. The core of a probabilistic model is the variability of the stochastic system, and once this variability model has been constructed the uncertainty of parameters can be overlaid. Uncertainty, or our lack of knowledge, as discussed in chapter 7, includes scenario uncertainty, model uncertainty, and parameter uncertainty.

Chapter 8 deals with model validation and review. It underlines that understanding the process used to develop the results can improve credibility of risk assessment results. Peer and public review of results is an essential part of the process. Interdisciplinary interaction is essential to the process of risk assessment, and should be extended to the review process. Experts in the biological processes involved should review the basic concepts and underlying assumptions used in an exposure model. Furthermore, statistical experts should review the data analysis and model construction. The public review process serves two main purposes. First, it allows all stakeholders in a risk assessment to critically review the assumptions made, and their effect on the risk assessment results. Second, it allows for evaluation of the completeness of the information and datasets used for the exposure model.

In chapter 9 and 10 the conclusions and recommendations are given. One main conclusion is that there is often no good match between the data available and the data needed for the exposure assessment. Data gaps and priority of requirements should be clearly communicated to the risk managers. Also the present limitations in the predictive microbiology models should be addressed. Further evolution of the methodology of quantitative risk assessment is needed. Therefore a quick harmonisation should be avoided.

The report addresses modeling exercises already done for processing and retails and handling in private households. It recommends that the possibilities for also using the modular processes approach for exposure assessment in primary production and consumption should be explored.

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1.Foreword.

The fast development within World Trade Organisation (WTO) has encouraged Codex Alimentarius to establish quantitative risk assessment regarding pathogenic microorganisms and develop tools to do so. The same demand is thereby introduced to the European Commission and its expert fora.

The quantitative assessment of exposures to microorganisms through food intake is an important part of the quantitative risk assessment made possible by the application of newly developed simulation approaches applicable to high performance computers.

It is the expectation that these tools eventually will be used to all kind of microorganisms entering the food chain either by purpose such as food fermentors or probiotics, or through contamination with pathogenic microorganisms.

So far most experiences in this new field have been obtained by modelling the behaviour of food borne bacterial pathogens in the food chain, and the working group decided to explore the application of the new principles and tools in risk assessment in this field before moving into other areas of food microbiology.

The previous report of SSC of October 26/27, 2000 stated (recommendations 14.8 and 10 pg 152) that priorities should include the development of guidelines for carrying out quantitative risk assessments and stepwise procedures for assessing exposures. One of the tasks would be to develop common exposure models and scenarios. Furthermore the report noted that the best assessment could be produced by a combination of epidemiological and risk assessment methods. The previous report also reviewed the risk assessments in use, and recommended that the possibilities for quantitative analyses should be explored wherever possible.

The report also identified several cases where QMRA could be helpful including food and drinking water, medicines or bio products, cosmetics, environment and water.

The WG decided to concentrate on the exposure assessment for microbiological risks for food borne pathogens. Food borne pathogens was chosen since several risk assessments on food borne has be published and been reviewed by the FAO/WHO activities the during the last years e.g., risk assessment of Listeria monocytogenes in foods and salmonella in eggs.

This report has been prepared for the SSC by a specially invited Working Group on Risk Assessment as applied to biological materials. The Members of the Working Group have been M.Cornu, E.Gallagher, A.Havelaar, A.Henken, I.Knudsen, R.Lindqvist, M.Nuti, B.Nørrung, F.O´Gara, P.Teufel and I.Vågsholm. Besides the Working Group Members a number of experts in the field, especially M. Nauta and B.Christensen are to be acknowledged for their contributions and advice during the process.

Abbreviations:

MPRM: Modular Process Risk Model
SIEFE-system: Stepwise and Interactive Evaluation of Food safety by an Expert System

QMRA: Quantitative Microbiological Risk Assessment

2. The process of exposure assessment in the context of risk analysis.

2.1 Introduction:

The risk analysis process consists of 3 interacting elements: *risk management, risk assessment* and *risk communication* (FAO/WHO 1997). The risk management driving the process can be described as a circle or as a helix type figure constantly improving by including information developed independently elsewhere e.g. the risk assessments (see fig. 2.1).

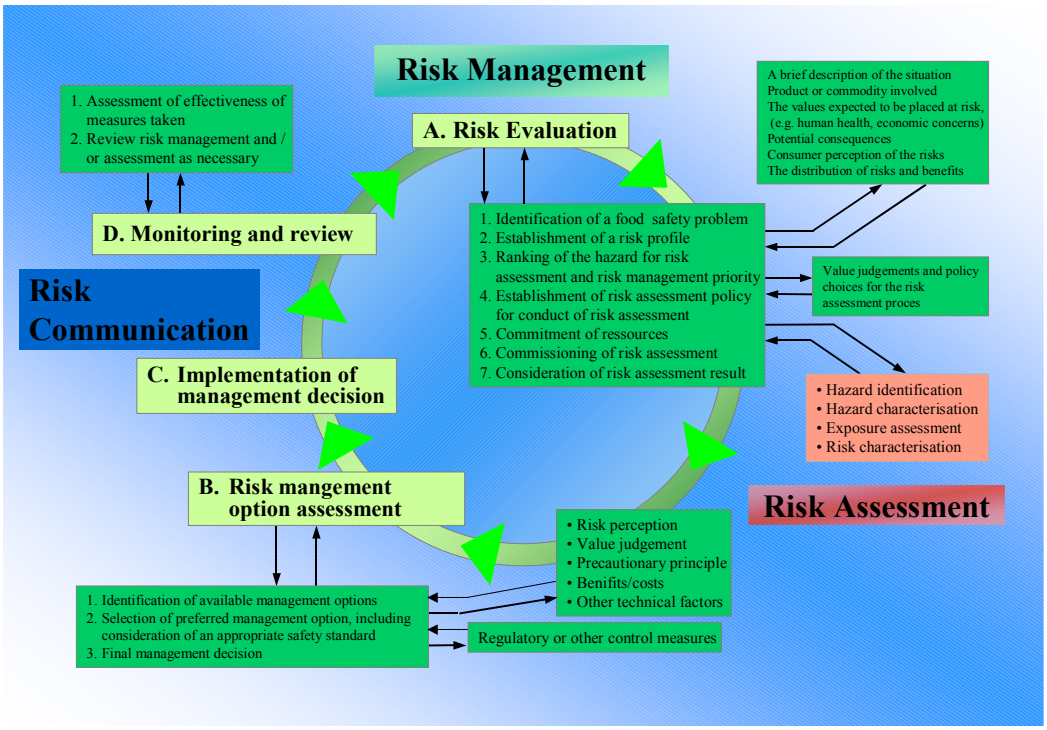

Figure 2.1. The Risk Analysis Process

As illustrated in figures 2.1 and 2.2 the processes of risk management and risk assessment are inter-linked and iterative in nature, and rely heavily on a successful risk communication ("sea of communication") between all parties involved.

To make the best use of the results of exposure assessments it is important to keep in mind the iterative nature of the overall process of risk assessment including the need for doing the initial preparatory work in the risk evaluation phase. In this phase it is important with an efficient dialogue between risk managers and assessors. For example since there are several possible definitions of an exposure assessment one should be clear what kind of definition to be used.

Moreover, in this preparatory phase it is crucial to clarify the purpose and scope of the risk assessment, i.e. the risk management questions as developed during steps 1-6 in the risk evaluation phase (Figure 2.1), that should guide the exposure assessment.

In this regard it is helpful to establish a risk profile as an initial evaluation of the issue with regard to the public health concerns, based on current state of scientific knowledge and the putative control measures available. In addition the risk profile is helpful when prioritizing issues to be dealt with.

The link from exposure assessment to risk characterization is established by combining the outcome of the hazard characterization and the exposure assessment process for an identified hazard as illustrated in figure 2.2. The purpose is to model the probability of disease in an individual at a particular occasion.

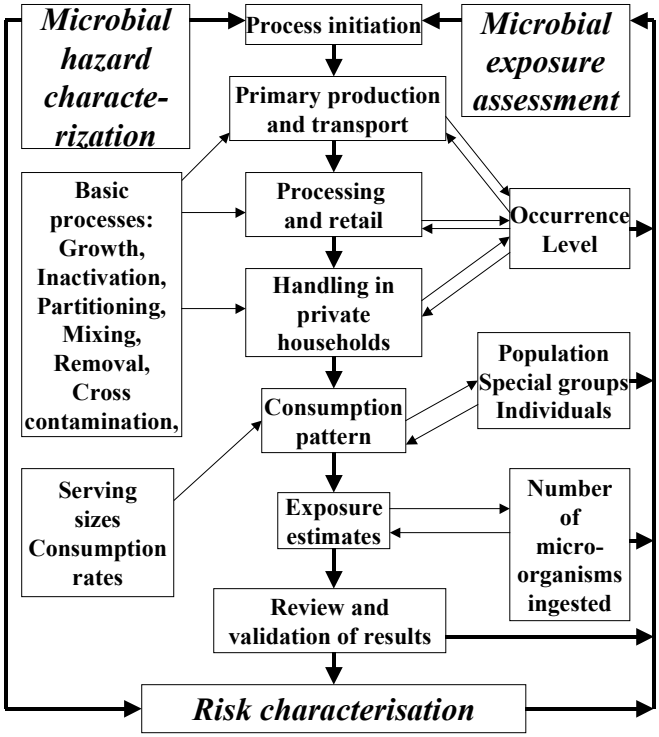

Figure 2.2. Exposure assessment of food born bacterial pathogens.

2.2 Definitions

Exposure assessment has been defined in several ways (Covello and Merjhofer, 1993; SSC, 2000). For the purpose of this document, the following definition appears to be useful: *Exposure assessment provides an estimate of how likely it is that an individual or a population will be exposed to a microbial hazard and what numbers of organisms are likely to be ingested.* (Lammerding and Fazil, 2000).

2.3 Purpose and objectives of the risk evaluation

Risk evaluation: A full quantitative risk assessment of a food safety problem is a resource and time-consuming enterprise. Only a few risk assessments has been completed to date for a few pathogen commodity combinations (WHO/FAO consultations). It appears that prioritizing quantitative or qualitative risk assessments that should be done is a critical risk management task. Thus, the pivotal step in the whole risk analysis process (see Fig 2.1) is the risk evaluation step where one identifies hazards, develops risk profiles, sets priorities and allocates resources; commissions risk assessments and evaluate their results. Risk evaluation is the front line of the risk analysis process, and is a risk management step. It could be argued that most of the resources available should be devoted to risk evaluation. It is in this step hazards are screened on a regular basis with the purpose to flag that a particular hazard deserves closer scrutiny. Moreover, when setting risk assessment policies including purpose and scope, a critical part is to formulate such questions to the risk assessors that can be answered. An ambiguous scope and purpose and vague questions tend to result in not always useful answers from risk assessments. In conclusion, a robust dialogue between risk managers and risk assessors is needed during the risk evaluation step.

The purpose and objective of an assessment should guide its analysis (Morgan and Henrion 1990). In order to clearly define the purpose and scope of the exposure assessment an understanding of the risk management questions is crucial, and a close interaction between managers and assessors is necessary during the initial phases this work could include the development of risk profiles, initial identification of putative hazards. 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. In general, the exposure assessment should be made as simple as possible while still including the important sources of risk.

2.4 Risk profiles

In order, to clearly define the purpose and scope of the risk assessment, including exposure assessment, an understanding of the risk management questions is crucial, and a close interaction between assessors and managers is necessary. During the initial phases this work should include the development of a risk profile.

Elaboration of a risk profile is essential for effective risk management. The risk profile should place the food safety issue within a particular food safety context and provide as much information as possible to guide further action. The risk profile should be carried out in collaboration between risk assessors and risk managers.

A risk profile provides an initial evaluation of the food safety issue in relation to the scope of the public health concerns, the extent of pertinent scientific information and available control measures. This can include and initial evaluation of the advisability and feasibility of conducting risk assessment.

For example a risk profile might optimally describe:

- which microbiological hazards are causing the problem and the difficulty in controlling them (nature and size of the problem, etc.);
- the source of the microbiological hazard from the entire food chain, including imported food, the environment, travel, animal contact and person to person transmission.

- An evaluation of to which extent the different sources contribute to the health problem.
- the available data on prevalence and numbers of the organism in question from the whole food chain;
- the disease incidence data and the types and severity of the adverse effects;
- which populations may be affected (for example, at risk groups such as the elderly, infants and children, the immuno-compromised, or those whose exposure to the microbial hazard may be increased due to dietary intake; socio-economic status, or other characteristics);The consumer perception of the problem;
- What is expected to be at risk (e.g.human health, economic concern);
- The available options;
- The potential consequences of action taken or which might be taken (including preventive measures);
- The distribution of risks and benefits.

2.5 Issues to be addressed in risk assessment including exposure assessment.

To achieve alignment between the risk assessment process and the needs of the risk managers it is necessary to clearly define the issues that the assessors should address.

The outcome and conclusions of the risk profile should enable the risk managers to decide whether a formal risk assessment should be carried out. Furthermore the risk profile should enable the risk managers to state the objective of the risk assessment and to define the specific questions that should be addressed. In this process effective communication between the assessors, the decision-makers and the stakeholders is crucial in order to ensure that questions asked by the risk managers could be answered and to ensure that the risk assessment provides the information needed by the risk managers.

Thus if the purpose of the risk assessment is to identify and compare different management tools to be used from farm to table then the entire farm to table pathway has to be addressed. In other cases only the pathways from retail to consumers are relevant for instance if the purpose of the assessment is to reach a decision on a regulatory proposal regarding the level of a pathogen in a ready-to-eat product to attain a specified level of protection with a high degree of confidence. In addition the level of detail required in the different pathways should be defined according to the information obtained in the risk profile. If, for instance, a risk profile has shown that the prevalence or numbers of a specific pathogen differs within a specific commodity dependant on i.e. type of slaughter house, type of slaughter processing, type of storage at retail level etc. such information might influence the details required in selecting the pathways in the exposure assessment. In addition the managers, despite the outcome of the risk profile, may have specific questions concerning specific processes i.e. organic farming, logistic slaughtering, imported foods etc. which they want to be addressed and which thus should be taken into account in selecting the pathways in the exposure assessment.

Whether the pathways wanted by the managers could be addressed will to some extent be determined by the available data. Whether data are available and to what extent they are sufficient should however be evaluated by the risk assessors.

The scope of the assessment may be limited to a specific product-pathogen pair or to several products or pathogens. Most often the scope are limited to a specific product-pathogen i.e. *E. coli* 0157 in steak tartare or Campylobacter in chicken meat.

If one is working with trade in or movement of animal and or animal products the assessment should follow the OIE international Animal health Code Article 1.3 (http://www.oie.int/eng/normes/mcode/A_00010.htm). It might be noted that although the Codex and OIE risk assessments are different in outline and heading the actual assessment job to be done is reasonably similar.

Once the purpose and scope of the risk assessment have been defined, the assessment should follow the framework identified by the Codex *Principles and Guidelines for the Conduct of a Microbiological Risk Assessment* (ALINORM 99/13A, Appendix II).

Defining the purpose and scope of a risk assessment is part of the risk evaluation. Apart from this a risk assessment policy should also provide documented guidelines for dealing with uncertainties, for value judgements or policy choices, and make provisions for apportionment of adequate resources, and for peer review. Risk assessment policy setting is a responsibility of the risk managers.

2.6 The links

Exposure assessment is an interdependent process where the links to the other part of risk analysis exercise should be clarified. Hence before proceeding to the exposure assessment it would be helpful to examine the links to the other part of the risk analysis process, for example:

- What are the relevant intervals of exposure in terms of dose response and observed levels of exposure?
- Does the dose response model capture all relevant facts?
- Are there special dose responses for vulnerable groups?
- What are the possible ways to limiting exposure?

Links to risk communication – Based on these considerations it appears that an open exchange of viewpoints and clarifications of questions is needed between stakeholders, risk managers, and assessors. This openness is needed for the analysis to produce relevant and useful results. Moreover, it could be hoped that the transparency would contribute to ensure that the results of the risk analysis are easier understood and accepted.

An important point is that the lack of knowledge and any missing pieces of information can be identified and dealt with a transparent way. An example is the extrapolation of a relationship between exposure and disease derived from an interval where data is known and the level of exposure where one wishes to make inferences about.

Links to risk characterization. The purpose of risk characterization is to integrate the information from hazard identification, characterization and exposure assessment to obtain an estimate of the of the likelihood and severity of the disease for an identified hazard. In other words one is trying to model unique events in a population that persons are exposed with sufficient pathogens to catch disease. Thus, the conclusions from risk characterization are valid on a population basis while they might be less valid for an individual. When doing exposure assessment is important to have a clear idea from the hazard characterization and the dose response curve about the relevant intervals for exposure in quantity and, where necessary, time period. Thus the hazard characterization and exposure assessment are linked processes.

The purpose is to model the unique event that the exposure is sufficient to cause disease in an individual at a particular occasion.

It is easy to think of quantitative exposure assessment as a prospective exercise where numbers are calculated to estimate exposures with given levels of probability. Thus, give certain levels of exposure one could predict an incidence of disease in the risk characterization exercise. However, these predictions should be compared whenever possible with epidemiological studies.

Thus, several strong assumptions may or may not be made in a risk characterization model. These assumptions underlying the risk assessment might include:

1. The individual has a consistent vulnerability for disease over time.
2. All individuals have the same vulnerability for disease.
3. The hazard is homogenously distributed in the foodstuff considered.
4. The hazard concentration or amount can be modeled over time with regard to multiplication, survival, temperature, pH, salinity and other relevant factors as the case may be.
5. The average and extreme exposure can be modeled or guesstimated at the time of the individual exposure (e.g., food consumption)
6. It is clear whether the exposure is cumulative (exposure to lead or cadmium) or unique for each occasion (microbiological exposures).

While this is an example list of possible assumptions to be made in a MQRA, the WG will emphasize that the risk assessment should handle the assumptions in a transparent way. The key issue is that one need to examine deviations from the stated assumptions of the analysis. Moreover, to check whether these deviations are critical for the conclusions drawn.

2.7 Discussions and conclusions

One should look upon the risk management as an iterative and continuous process. The first step of the management process, the risk evaluation, does include the development of a risk profile and may from case to case result in a recommendation that a formal risk assessment to be made by external experts. The pivotal step in the whole risk analysis process (see Fig 2.1) is the risk evaluation step, where one identifies hazards, develops risk profiles, sets priorities and allocates resources; commissions risk assessments and evaluate their results.

The risk assessment itself consists of four steps for food borne hazards: hazard identification, hazard characterization, exposure assessment and finally risk characterization with the final conclusions and recommendations where the hazard characterization and exposure assessment are linked processes. Risk assessments in other instances (e.g., trade in live animals) might have slightly different outlines.

The results of the risk assessment are used to guide the next step of the risk management process, the risk management option assessment and selection. This step is followed by the implementation of the management decision and later by monitoring and review of the success. This might lead to a new iteration depending on the success of the strategy used or the new pieces of information data triggering a new risk assessment. Hence, the risk management process is rather a structured way of dealing with risks rather than providing the final answer.

Here it is important to consider the epidemiological viewpoint on the black box approach. Black box epidemiology is when one is describing the relationship between risk factors and disease without a complete understanding of the biological mechanisms. The black-box approach is the logical consequence of that epidemiological research is at the cutting edge of medical research. As a consequence, one works with tools and concepts and biological systems that are not completely understood. Several arguments could be advanced for (Savits, 1994) and against (Scrabanek, 1994) the black box approach. Nevertheless, the lack of understanding is however, not an argument for avoiding the black-box strategy but rather to use it in a transparent way and to interpret the results carefully after scrutinizing them and the study design for the threats to their validity such as selection or confounding biases (Rothman and Greenland, 1998). One should be in particular hesitant to interpret the results of a black box model outside the observed intervals from where the model was derived. One example of a successful black box epidemiology is smoking and lung cancer where the findings has been unequivocal for a causal relationship while the understanding of the biological mechanisms whereby smoking cause lung cancer have been much slower to emerge. In risk assessments, the issue might be the same if one wishes to assess emerging risks.

It appears that for the purpose of this exercise it could be advantageous to define exposure assessment as a process with the objective to examine the exposures to risk agents and with the intended end result a determination or guesstimate of the probability of and the likely levels of exposure in the human population.

In a risk assessment it is also important to be explicit about assumptions made. These assumptions underlying the risk assessment might for example include that the individual has a consistent vulnerability for disease over time and that all individuals have the same vulnerability for the disease.

What may be a mild disease for robust adults can be life threatening for frail elderly persons, infants or persons suffering from some concurrent disease. Some food-borne agents such as *Listeria monocytogenes* may only give significant signs and symptoms to those individuals whose natural defences against these agents are deficient because of inherited or acquired immune depression (SCV 99). Another example is the increased susceptibility of people with iron accumulating disorders for *Vibrio parahaemolyticus* infections (SCV 2001).

Thus it should be made clear whether one does particular exposure assessments and risk characterizations models for each risk group or whether the risk characterisation model will apply to all risk groups. A risk groups could be subjects with AIDS, patients treated by cytotoxic chemotherapeutic agents and/or irradiation, and patients treated with glucocorticosteroids or immuno-suppressants for chronic systemic auto-immune diseases or to prevent organ rejection after transplantation. Malnutrition and various degrees of immune impairment make elderly another vulnerable group.

It should be emphasized that the risk assessment should handle the assumptions in a transparent way. The key issue is that one needs to examine deviations from the stated assumptions of the analysis. Moreover, to check whether these deviations are critical for the conclusions drawn.

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3. Model development

3.1 Introduction

According to Lammerding and Fazil (2000) exposure assessment is estimation of how likely it is that an individual or a population will be exposed to a microbial hazard and what numbers of the micro-organism are likely to be ingested. For exposure assessment 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, taking into account the variability and uncertainty attending this transmission (Nauta, 2001b). The food pathway mostly is split up into smaller steps. For each step, the input-output relation is then described. Essentially, this input-output relationship can be obtained either by observation (directly in the production process (surveillance)), by laboratory experimentation (simulation in the laboratory of the practical situation concerning certain specific steps) or by mathematical modelling (see e.g. Notermans et al. 1998).

The first method, i.e. *observation*, has the advantage that it is process-, product- and micro-organism specific, but the disadvantage is that it is usually time consuming and expensive, while also effects of various factors may be confounded.

The second method, i.e. *laboratory experimentation*, has the advantage that effects of various factors can be unravelled, that it may be more quick and relatively cheap in case only one or a few steps need to be investigated, but the disadvantage is that it may not be very precise and not specific for the process studied as only a few factors can be studied at the same time and away from the practical situation.

Increasingly, one therefore turns to the third method: *mathematical modelling*.

The advantage of models is that they force the researcher to arrange and organize all information available in a logical way which helps to define precisely the problem under study and facilitates exchange of knowledge. Models may be used for prediction when verified and validated, two processes which may require data from both surveillance and experiments. The disadvantage may be that the model may become unrealistic especially in situations where no information for verification and validation is available.

Quantitative risk assessment, in particular when using stochastic models, is a specialised task that requires skills in mathematics and statistics in addition to microbiological and technological knowledge. As a consequence, risk assessments are usually conducted in large, multidisciplinary projects. Building a comprehensive model may be resource intensive. The output of risk models is relatively complex, and in order to guide the risk assessment and interpret the results, risk managers need to understand the basic principles of modelling and concepts like uncertainty and variability.

It is important to realize that the purpose and objective of an assessment should guide its analysis (Morgan and Henrion 1990). They should tell us what part of ‘reality’ has to be modelled and to what detail this should be done. A model may therefore be considered a caricature of that part of reality we are interested in. An understanding beforehand of the risk management questions is crucial and therefore a close interaction between managers and assessors is necessary in the initial

phase of exposure assessment. It will often be necessary to limit the scope of the exposure assessment to be able to address the specific questions raised. In general, the exposure assessment should be made as simple as possible while still including the important sources of and steps leading to the risk of concern. Based on the outcome of the initial phase decisions regarding the approach to modelling (mechanistic or empirical, probabilistic or deterministic, dynamic or static, etc (Hurd and Kaneene 1993)) and the structure of the assessment model (which pathways, single or multiple models) can be made. In general, models should have a biologically plausible basis (Anonymous 2001).

3.2 Modelling in exposure assessment

In exposure assessment the probability and the likely levels of exposure in some defined part of the human population is determined. To accomplish this, the whole chain should be defined, with an initial stage (primary production in most cases, processing if in-factory post-processing contamination is the major source for introduction of the hazard, etc.), intermediary stages (processing, transport, retail, storage, etc.) and a final stage (consumption). At each stage, the steps or key activities (like transport, storage, preparation and cooking at the consumer stage) and the level of detail necessary should be decided upon. In this way one can gain insight into factors introducing or magnifying the risk along the food chain or into effects of strategies of risk management. However, if the primary goal only is to estimate the risk to a population from a certain food-pathogen combination, one could instead simply rely on statistical analysis of epidemiological data and information as close to the point of consumption as possible (Lammerding and Fazil 2000). Such an approach would be useful for risk ranking as well.

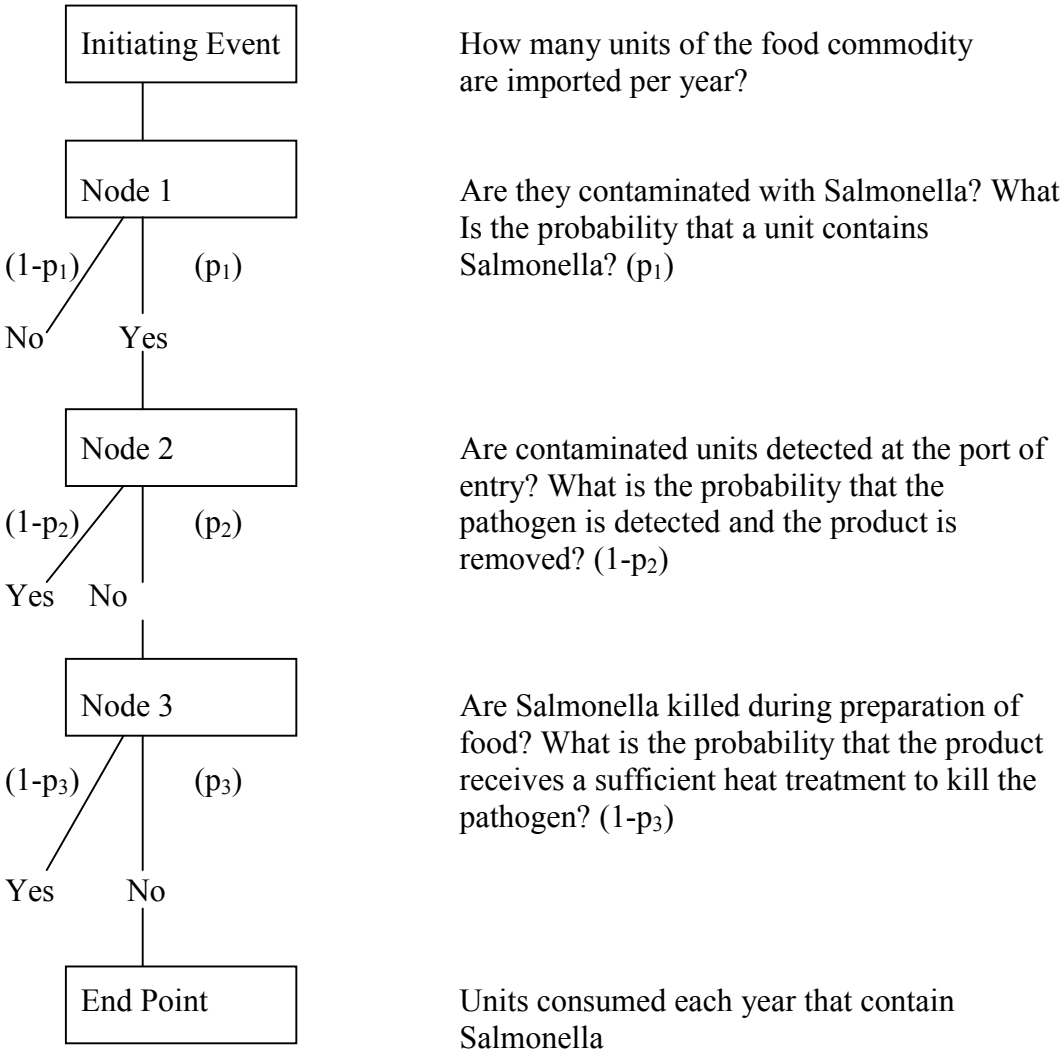
Estimation of exposure involves consideration of a number of complex and inter-related processes, describing the transmission of the hazard from the initial stage to the final stage in the selected food pathway, all of which contribute to the variability and uncertainty of the exposure. Given this complexity, it is often necessary to separate the overall chain into a number of distinct parts each representing a particular stage from production to consumption (Lammerding and Fazil 2000). Common stages to be recognised in exposure assessment can be identified. These may include primary production, slaughter & processing, handling & preparation, and even consumption, with likely transport and storage in between various stages.

Depending on the emphasis and the perspective of the exposure (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 end-point 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 (Figure 3.1a). In contrast to the Event Tree, the Fault Tree begins with the occurrence of a hazard (Figure 3.1b) 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. It should be emphasised that the type of

final quantitative model being used depends on the focus and perspective of the developer and on the problem being modelled. More elaborate and sophisticated classifications and distinctions between approaches and types of models can be done than in the present work (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

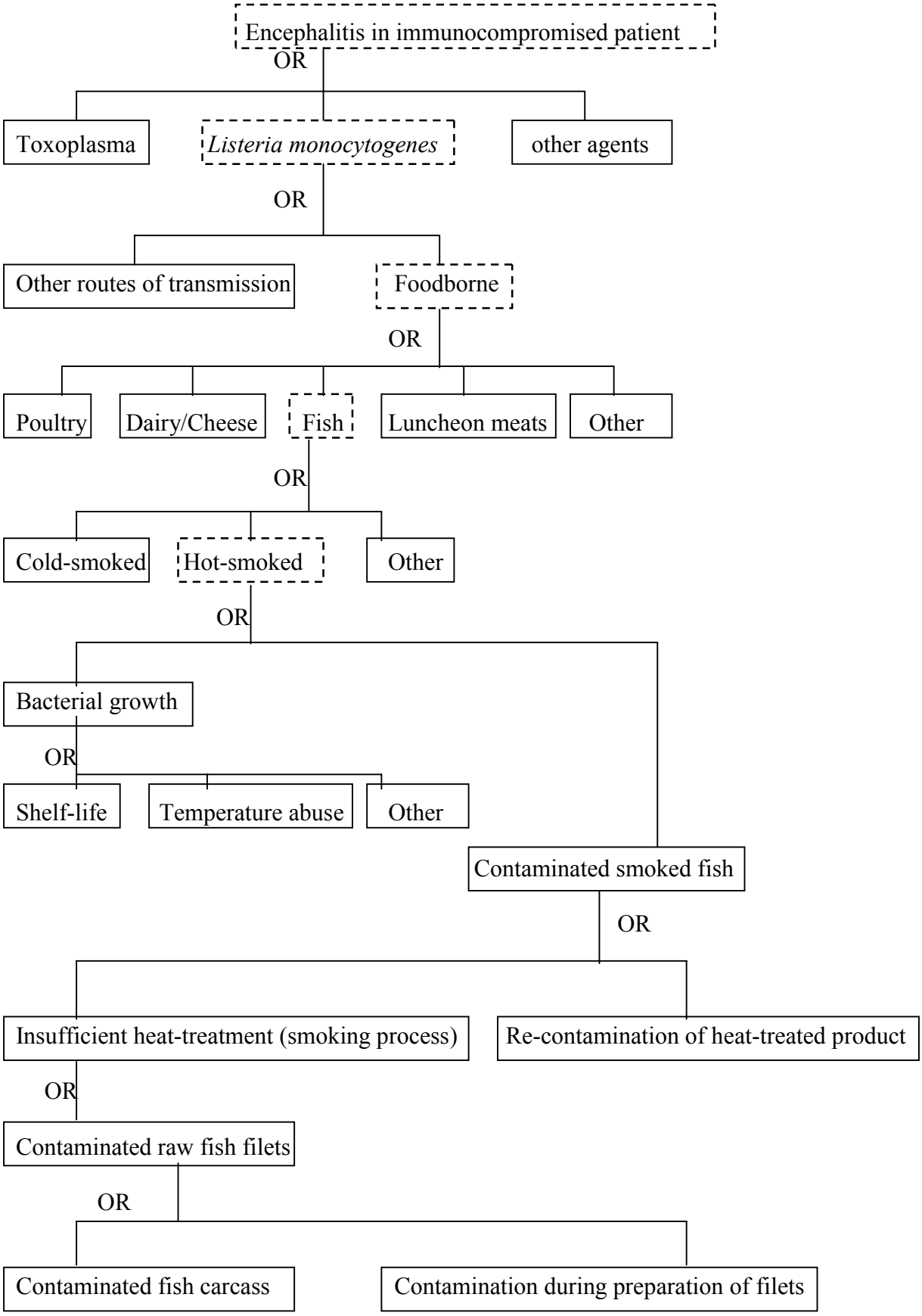
Figure 3.1a and b. Illustration of a simple Event Tree and Fault Tree, respectively. The event tree is a diagram of the events leading up to the end point of the risk assessment. An event tree predicts forwards. In contrast, the Fault Tree, which is a special type of event tree, begins with the occurrence of the hazard and from there moves backwards to identify the events that could and/or must have occurred for the hazard to be present. Fault Tree analysis is a method for analysing ways in which complex systems can fail, and for calculating overall failure rates from the individual component failure rates.

Figure 3.1a. Illustration of a simple Event Tree



p_{EP}= Probability of endpoint
F_{EP}= Frequency of endpoint

Figure 3.1b. Illustration of a Fault Tree



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). 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).

3.3 A general framework

A general framework for doing quantitative microbiological risk assessment (QMRA), the Modular Process Risk Model (MPRM) was recently proposed (Nauta 2001a, Nauta 2001b). The framework resembles the approach of the Process Risk Model (Cassin et al. 1998). At the heart of the proposal is the suggestion that to each of the steps or key activities at the various intermediary stages of a farm-to-fork chain at least one of six basic processes can be assigned, i.e. specifically, growth, inactivation, partitioning, mixing, removal and cross contamination. These basic processes are the six fundamental events that may affect the transmission of any microbial hazard in any food process. There are two 'microbial' basic processes, growth and inactivation, and four 'food handling' processes, mixing and partitioning of the food matrix, removal of a part of the units, and cross contamination. The 'microbial' processes strongly depend on the characteristics of the microbial hazard, as the effects of environmental conditions on growth and inactivation differ between species (and even between strains). Essentially, the effects of the 'food handling processes' are determined by the food handling process characteristics only, assuming a uniform distribution of micro-organisms over the food matrix. The MPRM focussing on the microorganism has not been developed for the primary production, where the animal itself is in focus.

The primary production stage therefore needs to be described in another way (e.g. by models designed to describe the dynamics of infectious diseases in populations of animals or plants). This is important to realise since several of the food borne pathogens are introduced into the production chain at the primary production stage. The output of such a description of the primary production stage would then function as input of the MPRM (initial prevalence, initial level of contamination (Figure 3.2)).

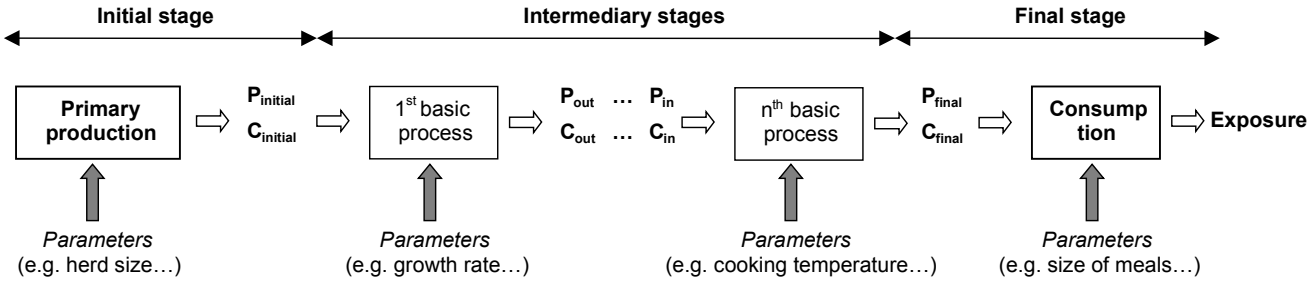


Figure 3.2: Schematic representation of a food pathway split up in different steps, each represented by an input-output basic process. P and C denote for prevalence and level of contamination.

The above would mean that a chain of n key activities can be described by at least n basic processes. However, while this may theoretically be true, it may in some cases not (yet) be possible

to allocate a basic process to a certain activity, because of insufficient understanding of what is exactly happening from a biological viewpoint. In such cases it is proposed to use a black box approach, as a last resort, by relying on an associative input-output description only. A sensitivity analysis of the model may indicate whether it will be worthwhile to unravel such a black box further. Alternatively, in some cases it may not be necessary or too complex in relation to the purpose of the exposure assessment to allocate a basic process to each activity. In these cases, it is proposed that several (consecutive) steps be considered together (aggregated) also described as a *black box model*.

In general, a model should be broken down into smaller components (disaggregated) as much as necessary to express significant logic between input variables and to model each uncertain variable as accurately as necessary for the efficient but accurate modelling in relation to the purpose of the assessment (Vose 2000). The more disaggregated such a model becomes, the more effort is required from the analyst and the computer in running the simulations and the analysis. The proposed framework (Nauta 2001a) can be summarised by the following seven steps some of which may have to be performed in an iterative process:

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

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

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

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

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

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

7) Perform an exposure assessment.

This proposal may form the basis for a discussion on a general framework for performing exposure assessments and QMRA. However, it should be realised that exposure assessment is limited by Nauta (2001a) to the level of contamination in the food product. The MPRM approach is developed to describe from a biological point of view the fate of hazard and food during what is indicated in Figure 3.2 as the intermediary stages. Consumption as such is not explicitly addressed in Nauta's report. As stated, the working group considers consumption to be part of exposure assessment. Since data on consumer behaviour are scarce simplifying assumptions often have to be made and this consequently will be a major source of uncertainty. The degree of sophistication that is required

for an exposure assessment is dependent on the degree of precision needed to adequately describe the behaviour of the microbe.

3.4 Basic processes

Nauta (2001b) proposed to calculate using numbers (N) of micro-organisms instead of concentrations (C). The advantage of using N instead of C in the calculations is that one is forced to do (realistic) calculations with discrete numbers, which is particularly relevant when N is small. So, for each step in the food pathway we are interested in the input-output relation for the number of cells per unit of product, *N*, and the fraction of contaminated units, the prevalence *P*. The definition of 'unit' is crucial. It is a physically separated quantity of product in the process, for example an animal, a (part of a) carcass, or a package of ground beef, a milk tank or a bottle of milk, etc. A relatively simple case is when a consumer package corresponds to one unit from primary production, typical examples being eggs (if not processed as egg-products) and chickens (if sold as full carcasses). Otherwise, units have to be redefined for each stage and at each mixing or partitioning. Preferably both *N* and *P* should be treated as uncertain and variable throughout the model, since then we will be able to assess the uncertainty and variability in the final exposure, and thus the uncertainty in the final risk estimate. Of course, knowing N and unit size one may calculate concentration by N/U which is the number of microbes per quantity (g or L or cm^2 or whatever).

Growth
The food borne microbial hazard is much more dynamic than the hazard in traditional chemical risk assessments because of the potential of some micro-organisms to multiply during various stages and in foods (Lammerding & Fazil 2000).

In general, a growth model has the structure (Nauta 2001b):

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

with N_{in} the number of cells at the beginning of the process, N_{out} the number of cells at the end of the process and $f(.)$ an (increasing, positive) growth function.

An empiric approach of the growth, based for example on the number of generations (Cassin *et al*. 1998) is possible.

The advantage of such an approach is that is relatively simple and that it requires data that are relatively easily available in literature. But it is essentially a black box model (Nauta 2001a) that does not explicitly link growth to the parameters that influence it: the nature of the matrix (pH, percent NaCl, water activity…), the temperature, the length of the storage time, and the behaviour of the specific organism under those conditions. This makes interpretation difficult.

Growth can also be modelled with tools of predictive microbiology, which aims to predict growth of pathogens in food over time as a function of different influencing parameters. For a review of such models see Van Gerwen & Zwietering (1998). Briefly, there are primary, secondary and tertiary models.

Primary models describe the evolution of a population of microorganisms over time under certain conditions assuming that these conditions remain stable during the period of concern. A large number of primary models has been developed and Table 3.1 presents the most common ones.

Model	Integrated equation	Differential equation
Exponential	$y(t) = y_0 + \mu\ t$	$\frac{dy(t)}{dt} = \mu$
Lag-exponential	$y(t) = \begin{cases} y_0, & t \le \lambda \\ y_0 + \mu \cdot (t-\lambda), & t > \lambda \end{cases}$	$\frac{dy(t)}{dt} = \begin{cases} 0, & t \le \lambda \\ \mu & t > \lambda \end{cases}$
Baranyi	$y(t) = y_0 + y_1(t) + y_2(t)^*$	$\frac{dy(t)}{dt} = \mu \frac{q_0 e^{\mu t}}{1 + q_0 e^{\mu t}} \left(1 - \frac{e^{y(t)}}{e^{y_{max}}}\right)$
Reparametrized Gompertz	$y(t) = y_0 + (y_{max} - y_0) \exp\left[-\exp\left(\frac{e^1 \cdot \mu}{(y_{max} - y_0)}(\lambda - t) + 1\right)\right]$	

$^*\ y_1(t) = \mu\ t + \ln\left[e^{-\mu t} - e^{-\mu(t-\lambda)} + e^{-\mu\lambda}\right];\ y_2(t) = \ln\left[1 + e^{y_{max} - y_0}\left(e^{\mu(t-\lambda)} - e^{-\mu(t-\lambda)}\right)\right]$

Table 3.1. Some primary models to describe microbial growth kinetics
With y(t): logarithm of concentration of the hazard (ln cfu/g); y_{max}: maximum population (ln cfu/g); y_0: initial population (ln cfu/g); q_0: initial physiological state (see Baranyi & Roberts, 1994); λ: lag time (h); μ: maximum specific growth rate (h^{-1}); t: time (h).

Whatever the equation, primary models are based on two main growth parameters: λ, the duration of the lag phase, and μ, the maximum specific growth rate. Taking into account different reasons (e.g., biological interpretation of parameters and/or equations, use of equation under non-constant environmental conditions), the lag-exponential function and the Baranyi model are preferable (Van Gerwen and Zwietering 1998).

As stated, in primary models it is assumed that environmental conditions remain stable during the period of concern. However, in practice these conditions vary in time and, consequently, bacterial growth will be affected. To include effects of non-stable environmental conditions secondary models were developed. Secondary models describe how the model parameters, λ and μ, vary with environmental conditions. Whiting (1995) describes three main types of secondary models: the response surface equation (multiple polynomial), Arrhenius relationship, and square root model (Bélehrádek). A comprehensive description of secondary model types is given by Ross and McMeekin (1994). Examples are presented by Van Gerwen and Zwietering (1998) and these are shown in Table 3.2.

Square root type models have biological interpretable parameters and the value of these parameters can be found in literature. Also the Arrhenius-Eyring (Schoolfield) model has biological meaningfull parameters, but the parameters are often used as fit parameters. The linear Arrhenius-Davey model and the polynomial model have no biological meaningfull parameters and rely purely on statistically based associations between input and output as determined by linear regression. A more detailed description of advantages and disadvantages of the various primary models is given by Van Gerwen and Zwietering (1998). As preference should be given to biologically plausible

Table 3.2. Secondary model types for growth rate in predictive microbiology (Van Gerwen and Zwietering 1998)
μ: specific growth rate (h^{-1}); T: temperature (degrees centigrade or, when indicated, Kelvin); a_w: water activity; pH: acidity; parameter$_{min\ or\ opt\ or\ max}$: this refers to (extrapolated) conditions at which no growth occurs or to conditions optimal for growth.

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 (Zwietering et al. 1996)	$\mu = \mu_{opt} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w)$ with $\gamma(T) = \left(\frac{T - T_{\min}}{T_{opt} - T_{\min}} \right)^2$ $\gamma(pH) = \frac{(pH - pH_{\min})(2.pH_{opt} - pH_{\min} - pH)}{(pH_{opt} - pH_{\min})^2}$ $\gamma(a_w) = \frac{a_w - a_{w\min}}{1 - a_{w\min}}$
Cardinal models (Rosso 1995, Rosso et al. 1995)	$\mu_{\max}(T) = \mu_{opt} CM_2(T, T_{\min}, T_{\text{opt}}, T_{\max})$ or $\mu_{\max} = \mu_{opt} CM_1(p\text{H}, p\text{H}_{\min}, p\text{H}_{\text{opt}}, p\text{H}_{\max})$ or $\mu_{\max}(T, pH) = \mu_{abs} CM_2(T, T_{\min}, T_{\text{opt}}, T_{\max}) CM_1(p\text{H}, p\text{H}_{\min}, p\text{H}_{\text{opt}}, p\text{H}_{\max})$ with $CM_n(x, p_{\min}, p_{\text{opt}}, p_{\max}) = \begin{cases} x < p_{\min} & , \ 0.0 \\ p_{\min} < x < p_{\max} & , \ \frac{(x - p_{\min})^n (x - p_{\max})}{(p_{\text{opt}} - p_{\min})^{n-1} \{(p_{\text{opt}} - p_{\min})(x - p_{\text{opt}}) - (p_{\text{opt}} - p_{\max})[(n-1)p_{\text{opt}} + p_{\min} - nx]\}} \\ x > p_{\max} & , \ 0.0 \end{cases}$

Arrhenius/Eyring (Schoolfield et al. 1981) *

$$\mu = \frac{\rho_{25}\frac{T}{298}\exp\left\{\frac{H_A}{R}\left(\frac{1}{298}-\frac{1}{T}\right)\right\}}{1+\exp\left\{\frac{H_L}{R}\left(\frac{1}{T_{1/2L}}-\frac{1}{T}\right)\right\}+\exp\left\{\frac{H_H}{R}\left(\frac{1}{T_{1/2H}}-\frac{1}{T}\right)\right\}}$$

Linear Arrhenius-Davey (Davey 1989) †

$$\ln(\mu) = a + \frac{b}{T} + \frac{c}{T^2} + da_w + ea_w^2$$

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.* (1981). T is temperature in degrees Kelvin.

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

models or models with biologically interpretable parameters square root type models would be the ones to choose instead of polynomial type models although the latter ones might fit statistically well.

Tertiary models combine primary and secondary level models with user-friendly application software or expert systems that calculate microbial behaviour under specified conditions. Two such user-friendly software packages that integrate both primary and secondary models are the American Pathogen Modelling Program and the British Food Micro Model, which are based on a Gompertz primary model and on polynomial secondary models.

Some authors used this predictive microbiology approach to take into account the impact of variability and uncertainty on parameters of primary or secondary models (Nauta 2000, Delignette-Muller & Rosso 2000).

Large progress is required for predictive microbiology to be adapted to the needs of quantitative risk assessment. The time dynamics of temperature are rarely taken into account, as realistic temperature profiles are poorly studied. Also new aspects forthcoming from progress of research have continuously to be taken into consideration (e.g., the phenomenon of quorum sensing). Moreover, secondary models do not enable to have a realistic prediction of lag times, especially after stressing conditions (such as those encountered by the bacterial population during the processing steps).

It is important to realise that a QMRA growth prediction has usually different demands than a 'traditional' predictive food microbiology growth model prediction. Whereas the latter is used to come to a growth curve prediction, that is a series of point estimates of growth for a time series, in a risk assessment model, especially for a controlled food production process, one usually has to predict both variability and uncertainty in growth after a fixed time period (see Figure 3.3). Predictive microbiology models are not developed for this purpose, and therefore one has to be

careful when applying them in a quantitative risk assessment study (Nauta 2001b, Nauta and Dufrenne1999).

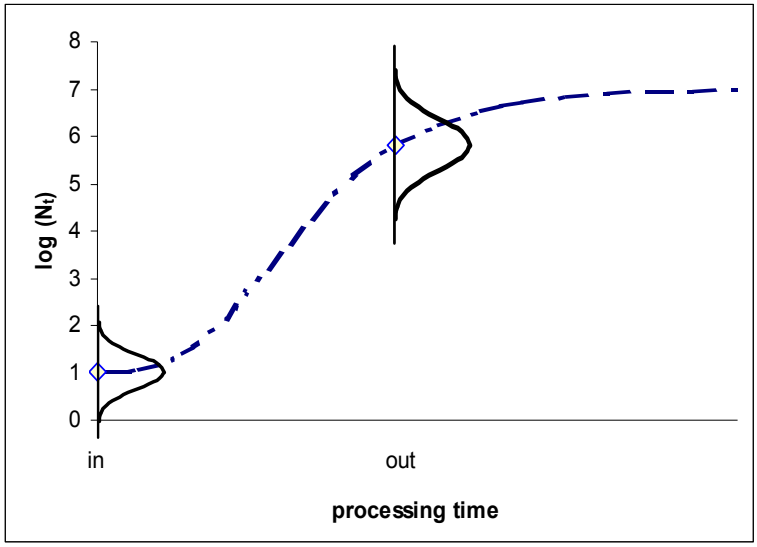


Figure 3.3. Growth is the increase in population size, given as log(N_t), as a function of time. Predictive microbiology models typically predict a growth curve, as given by the dashed line. In these models growth is considered as a function of time and the model yields a point estimate at any point of time t. In contrast, in QMRA we need a model that relates the probability distribution of the population size at the end of a process (out) to the probability distribution of the initial population size (in). Here, the end of the process may be at a fixed point in time. The probability distributions given by the 'bell curves' represent uncertainty and/or variability in population sizes N_{in} and N_{out}.

Inactivation

Microbial inactivation is the opposite of microbial growth. It is characterised by a decrease in the number of organisms per unit *N*. If the inactivation results in a decrease to zero living cells, the prevalence will decrease too.

The general formula for modelling inactivation is:

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

with *g*(.) an increasing (!) inactivation function. As for growth, many inactivation models are available (e.g.Van Gerwen and Zwietering 1998, Xiong et al. 1999. The most frequently used inactivation process is heating and the most frequently used inactivation model is the Bigelow model, in which inactivation rate is a function of temperature. It is linear in time (*t*) and has the shape $g(t) = t/D$, when the *D*-value (the decimal reduction value) at the process conditions is known. When D_{ref} , the *D*-value at temperature T_{ref}, and the *z*-value are known, the same inactivation function holds at temperature *T* with

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

Many of the aspects discussed for growth also apply for inactivation, e.g. existence of differences between strains of a species, or even of differences within a strain, due to adaptation (Nauta and Dufrenne 1999).

Partitioning
Partitioning affects the food matrix. It occurs when a major unit is split up into several minor units, as given schematically in Figure 3.4 :

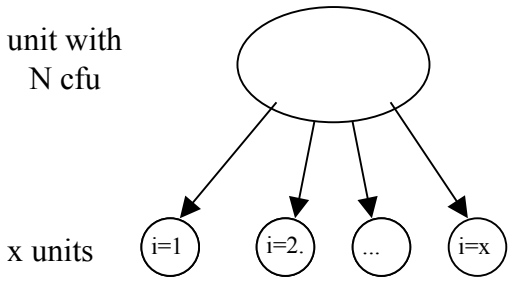


Figure 3.4. Partitioning: A major unit, containing N cfu (particles, spores, cells, etc) is split up in x smaller units i (i= 1..x). The problems to be solved are: (1) what is the number of smaller units with zero cfu and (2) what is the distribution of N_i, the numbers of cfu over the x minor units that are contaminated?

As stated Nauta (2001a) proposed to calculate with numbers instead of concentration. With the model we want to describe the distribution of the *N* cells in the major unit over the *x* smaller units (Figure 3.4). First, consider a possible change in prevalence. If, due to sampling effects, any of the smaller units contains zero cells, the prevalence will decrease. Assuming random sampling and equal sized units, the probability of an 'empty' smaller unit, P(zero cells in smaller unit) = P(0) = $(1\text{-}1/x)^N$, so the new prevalence

$$P' = P \times (1-(1-1/x)^N)$$

The expected number of empty smaller units is then

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

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

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

If P(0) is small (that is if *N* is large and *x* is relatively small), x_0 can be assumed to have a Poisson(E(x_0)) distribution. Then, the number of empty smaller units in a Monte Carlo model is a random sample from a Poisson($x^{1-N}(x-1)^N$) distribution.

Next, consider the distribution of the numbers of cfu per smaller unit. Assuming random distribution and equal sized smaller units, sampling leads to a number of cells N_i' as a sample from a Binomial (*N*, 1/*x*) distribution for one smaller unit *i* (hence the expected number of cells is *N*/*x*). If

the smaller units are not equal sized, and the smaller unit has size m compared to size M of the major unit, N_i' ~ Binomial (N, m/M) for this one smaller unit.

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

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

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

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

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

If the distribution of cells is not random, clustering effects can be taken into account by using a BetaBinomial distribution instead of a Binomial in the list given above, as shown by Nauta et al. (2001).

Mixing
Mixing also affects the food matrix and is the opposite of partitioning. In a 'mixing' process units are gathered to form a new unit, as shown in Figure 3.5.

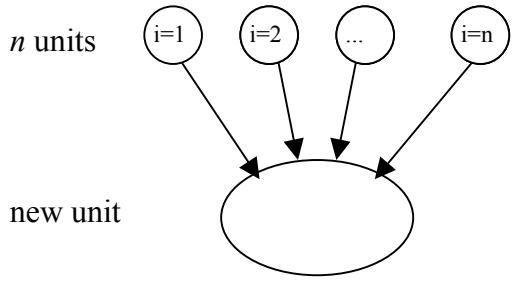


Figure 3.5. Mixing: n units, containing N_i cfu (particles, spores, cells, etc) in all n units i are put together to form a new larger unit. This larger unit will contain $N' = \Sigma_n N_i$ cfu. We want to know the distribution of N', given the distribution of N_i.

When n equal units are mixed to one, after mixing $P' = 1-(1-P)^n$ (assuming random mixing) and $N' = \Sigma_n N$. When differently sized units i are mixed, $P' = 1-\Pi(1-P_i)$, and again $N' = \Sigma_n N_i$.

These equations can be implemented directly into a spreadsheet model, but when n is large, this may be computationally inconvenient or even impossible. In that case you may use the central limit theorem as an approximation (see Nauta 2001a).

Removal
Removal is a process where some units (or parts of units) are selected and removed from the production process. Examples are the rejection of carcasses by veterinary inspectors in the slaughter house or the discarding of 'ugly' vegetables. If the removal would be a random process, it would have no effect on the risk assessment. However, the process is usually performed because there is a presumed relation with microbial contamination: (heavily) contaminated units are discarded more often than lightly or non contaminated units.

A graphical representation is given in Figure 3.6.

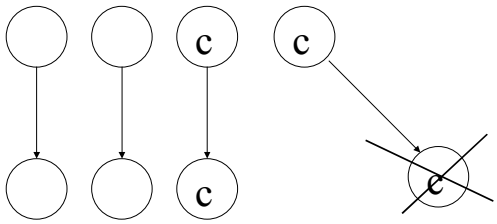


Figure 3.6. In the basic process 'removal' a fraction of the units is removed from the food pathway. Contaminated units (marked 'c') are removed with a higher probability than units that are not contaminated (not marked.) Typically, removal is a selection procedure based on e.g. visible effects of contamination.

Removal is often a subjective process in which the relation between rejection and level of contamination is obscure. Therefore useful mechanistic modelling of the removal process is complex. The simple model presented below is sufficient so far and may be improved in the future.

If it is assumed that heavily contaminated units are not discarded with a higher probability than lightly contaminated units, removal only affects prevalence, and not the variability distribution of the number of cells over the units. In that case removal can be represented by a factor f, such that the prevalence $P`$ after removal is equal to

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

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

Cross contamination
Cassin *et al*. (1998) proposed a factor for cross-contamination, F_{CC} in this equation:

$$P_{out} = P_{in} \frac{F_{CC}}{1 - P_{in} + F_{CC} P_{in}}, \quad F_{CC} > 1$$

This formula is identical to the one given above for removal, considering Fcc = f with f>1. The larger *f*, the larger is the impact of cross contamination. Like in the case of the removal process, this *f* may be variable and uncertain, derived from experimental results or expert opinion. Whether the effect of cross contamination on *N* is important, and how it is to be modelled, depends on the specific situation analysed.

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

Ignoring this last type of cross contamination, these cross contamination processes have in common that the prevalence is increased and that the number of cells remains about equal, and is only somewhat redistributed between the units.

Black Box
As stated above, some processes are to be or necessarily have to be regarded as black boxes as our understanding does not (yet) permit further unravelling in a biological meaning full way. A process like defeathering in a poultry slaughterhouse, for example, holds elements of removal, cross contamination and possibly involves growth or inactivation. If there is no proposal for an alternative scenario including this processing step, and the knowledge about its potential effects on the microbial hazard considered is limited, this might be regarded as 'one processing step'. The transmission is most easily modelled by linear models, that is by assuming that the number of cells changes by an uncertain and possibly variable factor: $N' = N.x$, so

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

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

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

The values of these factors will have to be estimated by data from the process considered or expert opinion. In both cases variability and uncertainty will have to be incorporated explicitly in the model. If there are series of input-output data available, these may show that the input output

relation is not linear. In that case another relation will have to be modelled, if possible with a mechanistic model, including relevant parameters.

3.5 Effect of basic processes on essential variables and data requirements

The qualitative impact on the transmission of each of the basic processes is given in Table 3.3. Note again that the columns in this table do not depict the concentration, but the total number of organisms (N_{tot}) and the 'unit size'. The prevalence (P , actually true and not apparent P in this case) in the table refers to the fraction of contaminated units.

The aim of the modelling of each of the basic processes is to describe the change in prevalence and number of micro-organisms per (contaminated) unit for each processing step, and this preferably in quantitative terms. For this, data will be needed on environmental conditions (e.g., temperature, pH) and (handling) practices (e.g., duration of transport, storage) at the various processing steps. To validate the model, data are needed on N, unit size and P at the start and end of all steps. Alternatively, in case N, unit size and P at the start and end of steps are known, environmental conditions may be estimated but then these subsequently need to be validated by measurements.

	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	+	= / +	=

Table 3.3. Basic processes of the MPRM and their qualitative effect on the true prevalence (P), the number of organism summarised over all units evaluated in one simulation run (N_{tot}) and the unit size. '=' : no effect,; '+': an increase; '-':a decrease.

Another category of data that is needed concerns the description of the food pathway. Although this may seem easy at first hand, experience showed that a description in quantitative terms (number of animals and their destination or their origin (national, import), number and weight of carcasses and their destination or their origin, etc) is not easily obtained. Moreover, when the model is to be used also to gain insight into risk factors and risk reduction scenario's, data on alternative food pathways and/or steps will be needed. In case risk may vary between different production systems or is subject of study (e.g., intensive (industrial) *versus* extensive (ecological) systems) data from such various different production chains will be needed.

A third category of data is associated with the definition of the population for which the exposure assessment is done. Exposure assessment should provide an estimate with associated uncertainty of the (variability in) occurrence and level of the pathogen in a specified portion of a certain food at the time of consumption in a specified population. It should therefore identify the food consumption

frequencies in a certain time period and the weights consumed in a given population or subpopulation and should combine the information to estimate the population exposure to the pathogen under study through the specified food commodity. Therefore data on amount and frequency of food intake in the given population or subpopulation is needed.

Apart from the challenge to obtain data for each pathway and step, another type of problem is associated with the fact that ideally data should describe the flow from source to consumption, i.e. data at step x should concern the same material as the data at step x-1 taken the basic processes in account. This however is seldom the case.

3.6 Discussions and conclusions

It is critical that the risk managers have clearly defined which questions or the scope and purpose the exposure assessment should address or focus on before the assessment is commissioned.

It is important to realize that the purpose and objective of an assessment should guide the analysis (Morgan and Henrion 1990). The purpose and the objective should tell us what part of 'reality' has to be modelled and to what detail this should be done. A model may therefore be considered a caricature of that part of the reality we are interested in. An understanding beforehand of the risk management questions is thus crucial and a close interaction between managers and assessors is necessary in the initial phase of exposure assessment. It will often be necessary to limit the scope of the exposure assessment to be able to address the specific questions raised. In general, the exposure assessment should be made as simple as possible while still including the important sources of and steps leading to the risk of concern. In general, models should have a biologically plausible basis (Anonymous 2001).

A preference should be given to biologically plausible models or models with biologically interpretable parameters. Square root type models would be the ones to choose instead of polymomial type models although the latter ones might fit statistically well. Inferences based on polynomial models should be limited to the data ranges from which the models were derived.

Large progresses are required for predictive microbiology to be adapted to the needs of quantitative risk assessment. The time dynamics of temperature are rarely taken into account, as realistic temperature profiles are poorly studied and primary models are not yet fully validated under non-isothermal conditions. Moreover, secondary models do not enable to have a realistic prediction of lag times, especially after stressing conditions (such as those encountered by the bacterial population during the processing steps).

It is important to realise that a QMRA growth prediction has usually different demands than a 'traditional' predictive food microbiology growth model prediction. The latter is used to come to a growth curve prediction, that is a series of point estimates of growth for a time series. In a risk assessment model, especially for a controlled food production process, one usually has to predict both variability and uncertainty in growth after a fixed time period (see Figure 3.2). Predictive microbiology models are not developed for this purpose, and therefore one has to be careful when applying them in a quantitative risk assessment study (Nauta *et al.* 2001, Nauta and Dufrenne 1999).

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4. Modelling the food chain.

4.1 Primary production models

The contamination or infection/colonisation of food producing animals, farm produce, fish/seafood with organisms pathogenic to man is well recognised as resulting in exposure of these pathogens to the human population. In human exposure assessment it might be necessary to model the stage of primary food production depending on microorganism in question and the scope of the risk assessment.

The use of probabilistic quantitative risk assessment (QRA) methodologies in the modelling of food-borne bacterial pathogens in primary food production is so far fairly limited and often exists as the first module of a 'Farm-to Fork' human exposure QRA focussing on the risk of infection from food producing animals. Such QRA's include the modelling of *Campylobacter sp.* in broiler chickens (Hartnett, in press), *Salmonella sp*. in pigs (Wong and Hald, 1999) and *Salmonella sp.* in egg production (FDA). Models assessing the risk of human exposure to farm produce and fish/seafood are more limited in number, for example, *Vibrio parahaemolyticus* contamination of oysters (FDA, 2001, this model is currently in draft form, therefore specific details will not be discussed).

In addition, the prevalence of Salmonella infection at various stages of the poultry production chain has been modelled with a view to evaluating intervention strategies (Nauta et al 2000).

The purpose of each of these models is to estimate the probability that a random animal from a defined group is infected with a particular human pathogen at the point of slaughter or the probable number of infected animals slaughtered per year. In the case of the FDA Salmonella in eggs model, the output is the probable number of Salmonella positive eggs produced per year.

In general terms, each of the models follows a similar risk pathway and each require similar input parameters. However the approaches taken in each model to estimate these parameters are often different and dependent on the type of data available. These approaches and the methods used to overcome deficiencies in available data are described briefly here.

The initial step in the 'Farm-to Fork' models was to estimate the prevalence of infected flocks/herds at a national level. This was achieved by estimating the total number of flocks/herds and the number of positive flocks/herds present at a national level.

Number of flocks/herds present
The QRA 'Farm-to Fork' models discussed here, each used nationally collected census data as denominator data.

The Campylobacter. model developed by Hartnett used nationally collected government census data to estimate the total number of flocks in Great Britain.

In the FDA Salmonella model, the numbers of flocks/herds were obtained from United States (US) National Agricultural Census statistics. The variability in the egg production in different sized flocks was taken in to account by stratifying the flocks by size again using US National Agricultural

Census statistics. This model also took into account the probability that there was more than one flock present on a particular farm.

In the Salmonella model developed by Wong, national surveillance data collected by the Danish Bacon and Pig Council was used to provide estimates for the national pig population in Denmark.

Flock/herd prevalence

Flock/herd level prevalence estimates the probability that a randomly selected herd/flock within a defined spatial area (eg country or region) is infected/contaminated with the organisms of interest.

In the absence of national surveillance data, flock/herd prevalence was estimated in the models using combinations of prevalence surveys, published data and industry data. In order to apply these relatively small scale survey's to a randomly selected animal at a national level it must be assumed that these flock/herd sampled in the survey is representative of the national flock/herd.

Biases in the survey sampling will occur as many of these estimates of prevalence are based on sampling of a sub-population of the flock/herd. Appropriate probability distributions must be used to describe these sub-populations and to extrapolate this prevalence estimate to the whole herd.

Furthermore the sensitivity and specificity of the diagnostic test used to estimate the prevalence is crucial and must be taken account of in the model. By not taking into account a sensitivity of less than 100% and underestimate will be calculated, whereas a specificity of less than 100% will result in an overestimate of prevalence. Appropriate probability distributions, for example the Negative binomial distribution, can be used to take in these cases (Vose, 2000).

In each of the models described here different methods of estimating flock/herd prevalence have been developed to take account of these problems in each of the particular data sets that were available.

The Campylobacter model developed by Hartnett used a combination of prevalence data of flocks supplied by poultry production companies, an epidemiological survey and published data. The estimates from each of these sources was modelled using a Beta probability distribution.

These estimates were then weighted according the relative proportion of the market they represented derived from government statistics.

The FDA Salmonella model uses two individual prevalence surveys of slaughtered spent hens as opposed to prevalence in laying flocks. An annual estimate of was obtained by combining these two surveys account for the seasonality of prevalence. Furthermore to account for the disproportionate geographical sampling this data was weighted by region based on National Agricultural census statistics. The surveys used in this model sampled 300 hens from each flock, therefore higher prevalence flocks were assumed to be more likely to be detected than low prevalence flocks. In order to account for this, the surveillance sensitivity was estimated and high prevalence and low prevalence flocks were modelled explicitly. A Beta probability distribution was used to estimate the proportion of the population that was positive.

The Salmonella model described by Wong uses seroprevalence data collected by the Danish Surveillance and Control Programme. An observed positive correlation between serological cut off

and the bacteriological status of pig herds was modelled by a probability distribution which was used a 'key' to translate new serological results into a probability of infection being present in a randomly selected pig.

Within flock/herd prevalence
The probability that an individual in a population is contaminated or infected may be described in by probability distributions or the use of dynamic population models.

The Campylobacter model developed by Hartnett describes within flock prevalence as a time dependant phenomenon for a positive flock. The model describes the transmission of Campylobacter within a flock using a complex combination of chain binomial and epidemic spread modelling in order to estimate within flock prevalence at a given time during the epidemic within the flock.

In comparison, the Salmonella model developed by the FDA estimates the frequency that Salmonella positive eggs are produced in each category of flock, based on published data which has been adapted for some flock categories. These frequencies were estimated using a Gamma probability distribution.

The Salmonella model described by Wong is more simplistic in nature estimates within herd prevalence using pig production data prevalence estimates described previously.

In general, surveillance programmes provide at group level (flock or herd) prevalence estimates, which are often determined by sampling of a proportion of the group, due to financial and logistical reasons. Often samples from each group are pooled for ease of testing. Evers and Nauta (2001) describe models which estimate an animal level prevalence specifically from pooled clinical samples. The models assume that part of the group is uninfected and allow for variation in the animal level prevalence within the group. The variation in the animal level prevalence is modelled in two ways.

The first model describes the distribution of animal level prevalence by three types of groups:
1. Group level prevalence *f1* with animal level prevalence *p1*
2. Group level prevalence *f2* with animal level prevalence *p2*
3. Non infected groups at group level prevalence (1-*f1*-*f2*)

$$P(x \setminus f1, f2, p1, p2) = (1 - f1 - f2)0^x = f1\binom{n}{x}(1 - q_1^m)^x q_1^{m(n-x)} + f2\binom{n}{x}(1 - q_2^m)^x q_2^{m(n-x)}$$

Where;
q1 = 1-p1
q2 = 1-p2
m = number of individual samples pooled into one sample
n = number of pooled samples in one group

The second model describes animal level prevalence using a Beta distribution:

$$P(x) = \int_{p=0}^{1} P(x \setminus p)g(p)dp$$

Where g(p) is the Beta distribution:

$$g(p \setminus \alpha, \beta) = \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} p^{\alpha-1}(1-p)^{\beta-1}$$

In each case the models developed here are pragmatic in nature and are primarily driven by the data available. In the absence of the data, appropriate assumptions have been made and some data have been modified in order to make them more appropriate to the area being modelled.

4.2 Processing and retail

Modelling of processing and retail may be the central module of a 'Farm-to Fork' human exposure QRA. Nevertheless, an assessment could also focus on characterising only the events that occur after processing (Lammerding & Fazil, 2000). Thus Lindqvist & Westöö (2000) have developed a quantitative risk assessment model in which the exposure and risk of acquiring listeriosis from consumption of packaged smoked or gravid salmon and rainbow trout were estimated. Growth or inactivation was not included in the model. In a risk assessment of ready-to-eat food, performed by FDA (2000), the starting point was at the retail level. None of the events occurring during processing or retail were then taken into account in those assessments. Such assessments may however be useful for risk managers to compare risks from different foods (risk ranking) or in the establishment of Food Safety Objectives.

All basic processes presented above (growth and inactivation, mixing and portioning, removal and cross-contamination) may occur during processing and retail, but they may be described using different ways.

Growth
In many studies, growth during processing is considered negligible, as the usual temperature in plant is below the minimum temperature of growth of the hazard. Nevertheless, Whiting & Buchanan (1997) took into account that one portion of units may be temperature-abused during this phase. Then, growth during transport and retail is more often taken into account. For exemple, Cassin *et al*. (1998) used the Food Micro Model to describe the post-processing growth of *E. coli* O157:H7 in beef, taking into account time on retail display and maximal retail storage temperature.

Inactivation
Reductions during process may be due to various treatments: chemical, mechanical, thermal…
Chemical and mechanical treatments are usually described empirically, for example by measuring the change in concentration during the process. The process can also be detailed into different steps, as in the Danish risk assessment on *Campylobacter jejuni* in chicken products (Christensen *et al*., 2001. A similar empirical approach, based on reductions (or log reductions), was used by Cassin *et al*. (1998), or Duffy & Schaffner (2001).

Reductions during process due to low temperatures (in particular freezing) are also often described empirically. But this step may also be considered without effect. For example, Brown et al. (1998) stated "the product has been kept frozen, and therefore the numbers entering the heat process are assumed to be the same as in the raw material". Nevertheless, Christensen *et al*., 2001 assumed that

the reduction during retail was null for chilled chicken but described empirically the reduction during conservation of frozen chicken.

Calculus of inactivation during a thermal processing (high temperatures) is generally based on a modelling approach, using tools of predictive microbiology, as described above (Whiting & Buchanan, 1997; Brown *et al*., 1998):

Cross-contamination
The hazard may be introduced into food during one of the processing (or even retail) steps by contact with equipment or by food handlers (Lammerding & Fazil, 2000).
If the direct contamination from primary production remains the main source of the hazard, the final prevalence could then be defined as a combination of the prevalence at the end of primary production and the probability of a cross-contamination during process or even retail.
The concentration of cross-contaminated units is often considered as a mere input parameter. But Christensen *et al*. (2001) proposed to describe concentration of Campylobacter in cross-contaminated units, by assuming that the hazard is diluted out as a function of the number of initially negative broilers processed after the positive flock. Thus, the first broiler (from a negative flock) entering the plant just after a positive flock would obtain a concentration similar to the final concentration of directly contaminated carcasses. After $t_{half,}$ broilers, the concentration would be reduced 50%.

Removal
Removal is a process where some units or subunits are removed from the production process, often because they are presumptively infected or contaminated. Meat inspection with identification and removal of infected animals is an example of this. Removal of Specific Risk Material in relation to BSE is another example.

Mixing
The mixing or blending of raw materials or ingredients can result in contamination of a larger volume of material, which can magnify the risk if the pathogen multiplies following mixing. Alternatively, if there is no multiplication, dilution of the hazard occurs when raw material is mixed with uncontaminated food. For example, a single lot of minced meat typically contains trimmings from several different animal carcasses; bulk tank milk is collected from different cows; broken eggs are combined before pasteurisation. These processes will dilute the hazard, when not all sources are contaminated (Lammerding & Fazil, 2000). Cassin et al. (1998) used a binomial law to describe the number of contaminated trimmings in a lot of meat, n being the number of trimmings in a lot and p the prevalence of contaminated trimmings.

Partitioning
Retail packages are usually parts of wide blends (milk tank, lot of meat, batch of liquid eggs…), which are split up into several minor units at one of the ultimate steps of the process.
To describe concentrations of the hazard in a portion v/V of a blend (v being the volume of the part and V the total volume of the blend), knowing the concentration of the blend C_{blend}, Bemrah et al. (1998) and Cassin et al. (1998) used Poisson distributions with $\lambda = v/V \times C_{blend}$. Whiting and Buchanan (1997) assumed a binomial distribution. These approaches assume independence and well mixing, i.e. random distribution.

The prevalence of contaminated packages is then modelled as the probability that a retail package contains at least one hazard (Cassin et al., 1998), or exactly one if the probability that a package contains more than one hazard can be neglected (Whiting and Buchanan, 1997).

4.3 Handling in private households.

When looking at pathways in private households from which individuals can be exposed to microorganisms, the situation may differ depending on whether the food is "ready-to-eat" or raw. Generally for foods bought at the retail level as ready-to-eat the storage conditions (time and temperature) may be the most important factors to take into account in the exposure assessment. Also for raw foods the storage conditions may offer possibilities for growth of certain microorganisms, which should be considered in the risk assessment.

In the home, individuals can be exposed to microorganisms through a large number of pathways during meal preparation using raw foods. These pathways could include: direct contamination from raw food to any food commodities not undergoing a subsequent cooking step before ingestion, indirect contamination of surfaces upon which cooked products or ready-to eat food are placed, contamination directly onto hands and subsequent ingestion, insufficient cooking and many other potential contamination events.

Possibilities of cross contamination in household have been modelled in relation to Risk assessment on *Campylobacter* in chicken products (Fazil *et al.* 2000, Christensen et al. 2001, FAO/WHO Expert consultation 2001). In the FAO/WHO Risk Assessment modeling of consumer handling and preparation in private kitchens has been divided into two parts: (1) Cross-contamination of a meal due to unsafe food handling procedures, and (2) The survival of *Campylobacter* due to insufficient heat treatment of the chicken.

Cross-contamination in the Home
Several investigations have been carried out to elucidate consumer habits during food handling in relation to cross contamination. In the joint FAO/WHO risk assessment on *Campylobacter* in broilers (2001) data on consumer habits related to washing hands after having handled raw meat and poultry and data on consumer habits related to cross-contamination by utensils are summarized.

Two models of cross-contamination have been developed. One describes exposure via fluid "drip" extruded from the uncooked broiler carcass and ingested by some pathway, e.g on fingers, or via contact with other foods. This model is a mechanistic approach related to the water a chicken gains through processing. Loosely attached cells will dilute into this fluid and may be inadvertently ingested. "The drip fluid model" used in the FAO/WHO risk assessment on *Campylobacter* in broilers is based on a model previously described in a Canadian risk assessment (Fazil *et al.,* 2000).

The second is a "contact transfer" model that quantifies the number of *Campylobacter* transferred from the raw chicken to preparation surfaces (cutting board, utensils, etc.) or hands, and subsequently from the preparation surface to a prepared meal or the organisms are directly ingested by e.g. licking on fingers. The "contact transfer model" is developed combining the modelling framework of a Danish and English risk assessment. Both risk assessments have used data from a study by Zhao *et al.* (1998) where the fraction of organisms transferred from a contaminated raw chicken to a cutting board, and further from the cutting board to salad was reported. Although data

were based on the organism, *E. aerogenes,* they may give a good indication of the possible maximum fraction of microorganisms to be transferred from one surface to another.

In models the extent to which each of the many possible contamination pathways may contribute to the overall exposure of humans is not considered, and with the current data available this is probably not possible. In reality both modelling approaches takes all possible cross contamination pathways into account in a single function. A Comparison of the two models shows that despite the different approaches taken mathematically they seem to be equivalent with an appropriate choice of assumptions. The resulting transfer from raw chicken to an exposure dose can be described by a single probability distribution F_t that describes the probability of a bacteria (agent) being transferred from the chicken to the meal during the preparation of a meal.

$$N_m = Binomial\ (N_c, F_t)$$

where N_m and N_c indicate the number of cells in a meal and on the raw chicken, respectively.

In the determination of the distribution, F_t, one of the two models may become preferable in future. However, this will probably depend on the nature of data that will become available.

In conclusion, the current models may indicate that with the information available it is difficult to become very detailed in relation to modelling cross contamination. Further improvement of the models and model validation may become extremely difficult given the complexity of the many different possible cross contamination pathways and the variability in the behaviour of individuals in the kitchen.

G*rowth*
With respect to most other organisms than Campylobacter the possibility of growth of the organism during long storage and/or wrong storage conditions is an extremely important parameter. Growth of microorganisms in private kitchens has been considered in a large number of exposure assesments: *Bacillus cereus* in pasteurised milk (Delignette-Muller & Rosso, 2000) or in broccoli puree (Nauta, 2001), *Salmonella* Enteritidis in pasteurised liquid eggs (Whiting & Buchanan, 1997), *Escherichia coli* O157:H7 in ground beef (Cassin et al., 1998) or in apple cider (Duffy & Schaffner, 2001), ...Such analyses are based on length and temperature of storage in the home fridge and microbial data regarding growth in these conditions.

Growth of microorganisms in a collective catering establishment has also be taken into account Bemrah et al. (in press).

Cooking
It is recognized that there are a variety of approaches to cooking food. Once an appropriate modelling approach has been determined for one type of cooking, the model can be extended to other cooking styles.

Inactivation during a thermal process uses the decimal reduction time, D, as an inactivation parameter. Different microorganisms may have different D-values, and this parameter could be measured, or taken from literature, for the microorganisms of consideration. In the literature several different D-values may be found for the same organism and since D-values are dependant on both the chemical and physical properties of the food (i.e. water activity, fat content, pH, inhibitory

substances etc.) and the state of the microorganism (log phase, stressed etc) it should be carefully considered whether a given D-value can be used in a specific exposure assessment.

In the FAO/WHO Risk Assessment three different modelling approaches have been considered. These were all related to the inactivation of Campylobacter during cooking. However, modelling wise they may apply for any given microorganisms.

In the first approach, referred to as the internal temperature approach, a representative point is chosen in the mass of the chicken and it is assumed that this represents an area of the chicken that can be expected to receive the mildest heat treatment. The reduction in the number of cells at this point is then calculated by calculating a step-wise time-temperature profile and applying reductions at each time-step. The variability in consumer practices is implemented in this approach by varying the stopping point (i.e. the final temperature) in the simulated time-temperature profile. The variation in the stopping point is based on survey data measuring this final temperature.

The second approach, referred to as the Protected Areas Approach, is based on the designation of areas in the carcass where the least heat treatment is achieved, presumably due to increased thermal insulation from the heat source. In this approach, it is assumed that any *Campylobacter* outside these designated areas are killed. The approach then requires assumptions regarding the proportion of pathogens that are found in these areas, and the maximum temperature achieved in this area. The thermal inactivation is estimated by D-value calculation for the final temperature in this area and the assumed time spent at this temperature.

The third approach, referred to as the Heat Transfer Approach, is designed to predict the time-temperature profile at various depths below the surface of chicken based on a simplified thermodynamic model of heat transfer through chicken meat. This allows for prediction of thermal inactivation as a function of depth and time. The final consumer exposure is highly dependent upon the final temperature achieved at each depth and the assumed proportion of cells which are located at each depth in the carcass.

Each of the three models has advantages and limitations and as for cross contamination models the model of choice will strongly depend on the data that will become available in future. At the current state, the models are being evaluated with respect to the validity of the required assumptions, the degree of conservatism, which is implicit in the approach, and the relative value of complex versus simple models given the amount of uncertainty in the location of cells in or on carcasses with respect to thermal insulation.

The importance of insufficient heat treatment related to human exposure will depend on the location of microorganism on a given food item and if the microorganisms are only located at the surface of the food undercooking may play a minimal role.

Additional considerations: Hygiene practices of food preparer
Since the hygiene practices is mainly related to the person preparing the food it may be considered to include variation in preparation practices among food prepares. Such an approach was used in the Danish Risk Assessment on Campylobacter in Chicken (Christensen et al.2001), where the number of people preparing food in different age and sex groups was related to data from telephone interviews in which the ratio between people with safe and unsafe food handling in different age and sex groups was obtained.

Table 4.1.a. Data on consumer habits related to washing hands after having handled raw meat and poultry (From the Joint FAO/WHO Risk Assessment, 2001).

Statement	**Respondents agreeing with the statement (%)**	**Study performed in**	**Reference**
Washing hands not performed after handling raw meat and poultry	34% of 1620 persons	US 1992-1993	Altekruse *et al.,* 1995
	18.6% of 19356 persons	US 1995-1996	Yang *et al.,* 1998
	55.8% of 1203 persons	Australia 1997	Jay *et al.,* 1999
	36% of 15 households	Denmark 1998	CASA, 1999
Washing hands not important in relation to food hygiene	18.4% of 1203 persons	Australia 1997	Jay *et al.,* 1999
Personal hygiene (inc. washing hands) not important for prevention of food-borne disease	62% of the 990 persons	Denmark 1996	AIM Nielsen & Levnedsmiddelstyrelsen, 1997
Drying of hands performed after hand wash	70% of 15 households	Denmark 1998	CASA, 1999
Observation	**Households where the observation was done (%)**	**Study performed in**	**Reference**
Washing hands not performed after handling raw meat and poultry	58% of 108 persons	UK 1996	Worsfold *et al.,* 1997a; Griffith *et al.,* 1998
	57% of 106 households	US and Canada	Daniels, 1998

Table 4.1.b. Data on consumer habits related to cross-contamination by utensils (From the Joint FAO/WHO Risk Assessment, 2001).

Statement	**Respondents agreeing with the statement (%)**	**Study performed in**	**Reference**
Knifes and cutting boards not cleaned in warm water + soap after handling raw meat and poultry and before cutting vegetables and salads	46% of 865 responses	US 1990-1991	Williamson *et al.,* 1992
Cutting board not washed after handling raw meat and poultry	33% of 1620 persons	US 1992-1993	Altekruse *et al.,* 1995
	19.5% of 19356 persons	US 1995-1996	Yang *et al.,* 1998
The kitchen facilities not sufficiently cleaned to avoid cross-contamination	11.6% of 1203 persons	Australia 1997	Jay *et al.,* 1999
Food items handled on not sufficiently cleaned cutting boards	25% of 108 persons	UK 1996	Worsfold *et al.,* 1997a; Griffith *et al.,* 1998
Meat and poultry packing material stored in the food handling area	18% of 108 persons	UK 1996	Worsfold *et al.,* 1997a; Griffith *et al.,* 1998
Food items handled in a way that could lead to cross-contamination	76% of 106 households	US and Canada	Daniels, 1998

As for the private kitchens the same hygiene practices may account for in restaurants. The consequence of preparing many chickens at the same time of which only some may be contaminated however should be taken into consideration.

At present there are no models concerning food handling in restaurants available.

4.4.Consumption patterns.

The previous section described the risk related to the behavior and hygiene habits of the person preparing a meal, but did not take into account the habits and food preferences of the actual persons ingesting the meal. As the frequencies and quantities of food items ingested for different population groups may differ significantly this may be extremely important in understanding the outcome of a risk assessment.

Depending on the purpose of the risk assessment, the population may be divided into sub populations in different ways. Subpopulations could be chosen according to geographic location (e.g. urban versus rural), age, sex, religion, races and education. In addition, it could be of great value to follow changes in consumption patterns over time either for the entire population or for different subpopulations.

The factors of relevance when detailing consumption patterns are the sizes of meals ingested, the frequencies by which different food items are ingested, preferred preparation styles, and finally the frequencies by which the food ingested is prepared at home, in restaurants, fast food restaurants, caterings, etc.

Data on the sizes of meals for different subpopulations may be obtained from national dietary surveys. This was for example the procedure in a risk assessment on *Campylobacter* in chicken products by Christensen et al. (2000) who used consumption data on the sizes of chicken and salad meals. Such data are essential for determining the dose of organisms ingested by an individual. For example, on the average the elderly and children ingest smaller sizes of chicken meal and is therefore also exposed to lower doses than e.g. young adults. On the other hand this may be counteracted by the fact that the elderly and children often are more sensitive to the pathogens than other age groups.

Another factor of importance is the frequencies by which different food items are ingested, which can be used to determine the average probability of exposure of pathogens via different food sources. Again such data may be obtained via national dietary surveys, or alternatively be calculated on the basis of the registered retail sale. For the latter such information is published on the FAO Internet home page (/http://apps.fao.og/page/collections?subset=nutrition).

Preparation style is of particular relevance in relation to beef and fish, which are often preferred raw or undercooked. In a quantitative risk assessment for Escherichia coli O 157 in ground beef hamburgers and another in steak tartare patties (Cassin et al., 1998, Nauta et al., 2001) the population was divided into different cooking preferences going from those who prefer rare to those who prefer well done meat. In both studies this information was used to determine the number of cases where the meat reached a certain internal temperature during cooking, which were then used to calculate the number of E. coli O157 cells that would survive (the dose) the cooking procedure.

Finally, the risk associated with the consumption of a given food item should be linked to the person(s) who prepare the meal. In private households, restaurants, catering etc. the hygiene level during preparation will affect the probability of being exposed to a contaminated meal. However it is important to emphasize that in addition to the hygiene level, the number of contaminated servings will also depend on the number of persons that the meal is prepared for. By merging population data from Statistics Denmark with data from a Danish dietary survey Christensen et al. (2000) could obtain a relationship between the person who prepares the meal and the persons ingesting the meal in private households. It was shown that if for example the person that prepare the meal belongs to the age group of 18-29 years (with a relative low hygiene level) the average family/household size is lower than for people in the age group of 30-65, which has a higher hygiene level. Thus, although the probability of illness per serving may be higher in households where a young adult prepare the meal as compared to the mid age group (30-65 years) the number of people being exposed may be lower because of a much smaller average family size in these households.

In a similar way, the consumption patterns may be linked to the preparation hygiene in restaurants, fast food restaurants and caterings. It may be expected that e.g. young people more often eat at fast food restaurants than elderly and that catering food often is prepared for older people. However, at present no QRA models details the hygiene practice in such places.

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5. Examples of modelling exercises.

This chapter presents some examples of modelling exercises that have been carried out in different European countries in recent years. The examples and accompanying text have been chosen to illustrate the principles outlined in the previous paragraphs, and not to fully represent the risk assessment studies themselves. For more detail, the reader is referred to the original publications. Each summary provides an introductory text to the model (based on the abstract of the report or publication), a flowchart of the food chain that was considered, and a table of steps in the food chain with the relevant basic processes.

5.1. A Shiga toxin producing *Escherichia coli* O157 in steak tartare patties.

The objective of the study presented in this paragraph was to further develop the methodology for conducting a quantitative microbiological risk analysis (QMRA) 'from farm to fork and beyond', to discover potential pitfalls and to explore the available data and their suitability for QMRA (Nauta et al. 2001, available via www.rivm.nl). It integrates 'farm to fork' exposure assessment modelling with microbiological dose response modelling. In this paragraph only the exposure part will be dealt with. The hazard considered is Shiga toxin producing *Escherichia coli* O157 (STEC O157), a notorious pathogen. Cattle are generally considered as the most important reservoir of STEC O157. Therefore, a beef product was chosen as the food product for which the risk is evaluated. Based on consumption frequency, potential risk and relative simplicity of processing, the product choice was 'steak tartare patties', a lean ground beef product, typically eaten raw or partially raw. To limit the complexity of the assessment, only the Dutch population and only data on Dutch animals and slaughterhouses were considered in the analysis.

As slaughter practices may differ, three routes of exposure were compared, separating 'industrial' and 'traditional' ways of both slaughter and subsequent processing (see figure 3.2.A and table 3.2.A). Consumers were separated in three age classes, 1-4 years, 5-14 years and 15+, to fit with the effect modelling. Next, three preparation styles of the steak tartare patties (raw, medium and well done) were considered. (Dutch) data were collected on the prevalence and concentration of STEC O157 at the different stages in the food pathway: farm, slaughter, retail and consumer.

When the food pathway was modelled, it appeared that important information required to estimate the values of the model parameters was lacking. Therefore an expert elicitation workshop was organized to estimate the values of the remaining parameters for which no data were found. One of these parameters is for example the faecal contamination of carcasses, expressed in gram faeces per carcass. The model was implemented in an @Risk spreadsheet and Monte Carlo simulations were run.

The exposure model predicts that about 0.3% of the raw steak tartare patties is contaminated with STEC O157. Of these contaminated patties, a large fraction (>60%) is contaminated with one

colony forming unit (cfu) only. High contamination levels are rare, with for example only 7% of the contaminated raw steak tartare patties containing more than 10 cfu. In a microbiological survey it was found that one of 82 raw steak tartare patties (1.2%) was positive for STEC O157. Knowing that the probability of detection of single cfu's in such a survey is small, this suggests that the model prediction is an underestimation of the actual level of contamination of steak tartare patties.

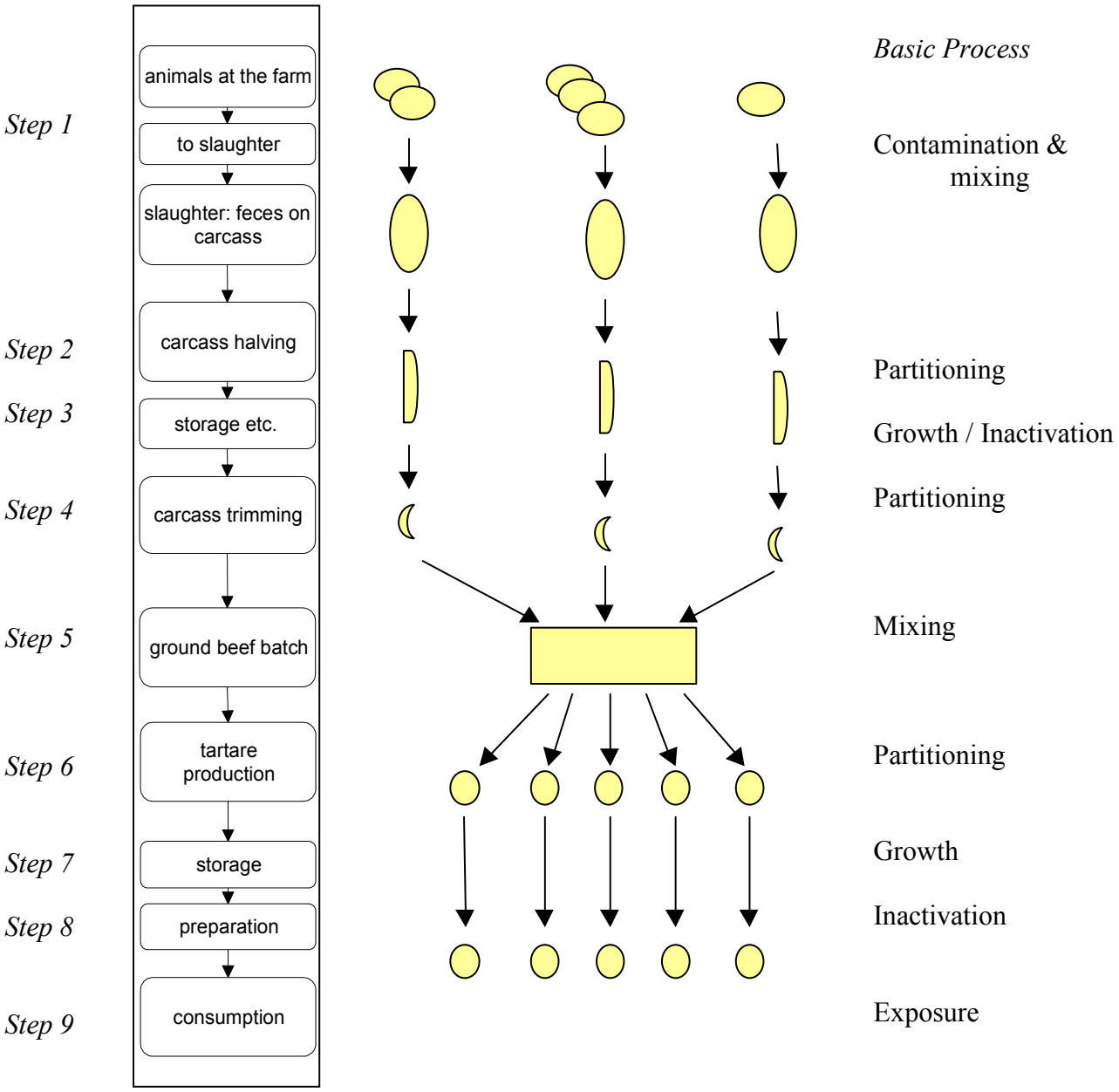


Figure 5.1. Food Pathway of steak tartare. The pathway is split up into nine modelling steps. The illustration shows how the units are formed by partitioning and mixing. As much as possible, a basic process is assigned to each of the steps.

The 'farm to fork' exposure model was linked to a dose response model of STEC O157, based on data of an outbreak in an elementary school in Japan in 1996. Results of this linkage will not be presented here. However, the ultimate risk estimate was highly uncertain. Nonetheless the risk model can be used to investigate the impact of uncertainties in the model input on the model output, and to evaluate intervention at different stages along the food pathway. Analysis of alternative scenario's shows that the uncertainty in prevalence and concentration of STEC O157 at farm level may have a large effect on the final model estimates. The same holds for uncertainty about growth and inactivation of STEC O157 on the carcass. In contrast, the effect of growth of STEC O157

step	*Process*	Unit	Description
1	*Start / mix.*	carcass	As input the model requires the prevalence of STEC O157 contaminated animals at slaughter, and the concentration in the feces. In the slaughterhouse a carcass is (potentially) contaminated by a mixture of feces from several animals. Several carcasses may contribute to the beef in one ground beef batch (in step 5).
2	*Part.*	half carc	Carcass halving is a partitioning process
3	*gr./ina.*	half carc	On the carcass, STEC O157 may grow or be inactivated during carcass handling and storage.
4	*Part.*	Trimmings	Meat is cut from the carcass for tartare production.
5	*mix.*	gr. Beef	Trimmings (from one or more carcasses) are mixed and grounded as a batch.
6	*Part.*	Tartare	Steak tartare patties are produced from the batch.
7	*Gr.*	Tartare	STEC O157 may grow during storage, depending on storage time and temperature.
8	*Ina.*	Tartare	If the steak tartare patty is cooked, STEC O157 will be inactivated.
9	*Exposure*		The patty is consumed by consumers from three age classes.

Table 5.1. The food pathway split up in steps, as illustrated in figure 5.1. A basic process is assigned to each step (part. = partitioning, gr. = growth, ina. = inactivation, mix. = mixing). The unit is defined (carc = carcass, gr beef = ground beef batch). A short description is given of each step. For model equations, see Nauta et al. (2001).

during retail and domestic storage is negligible and the effect of advocating the consumption of 'well done' steak tartare patties is questionable. This suggests that intervention at farm level or at slaughter is more likely to be effective as a strategy to reduce STEC O157 associated risks than intervention at the consumer level.

Performing the risk assessment has been a valuable experience. The MPRM was a useful approach. In the future, the focus of QMRA 'from farm to fork and beyond' should first be on a manageable statement of purpose and a description of the food pathway making an inventory of data needs, before surveying the available data. The potential discrepancy between model complexity and data availability should be carefully considered. Also, developing expertise in expert elicitation is recommended.

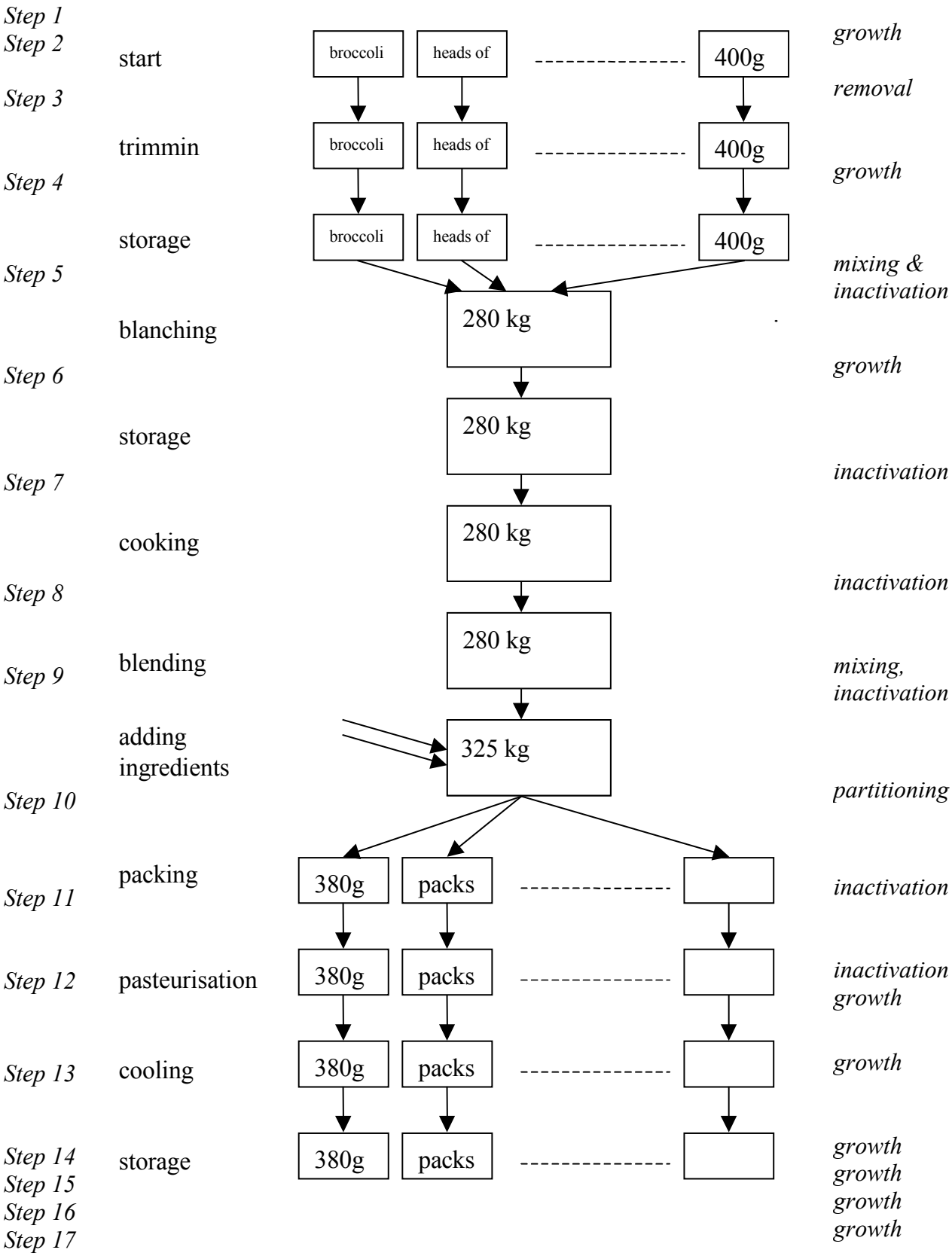
5.2 *Bacillus cereus* in broccoli puree.

The Modular Process Risk Model (MPRM) methodology is illustrated in a case study, an exposure assessment of a spore forming pathogen, *Bacillus cereus,* in a refrigerated processed food of extended durability (REPFED): a package of broccoli puree (see figure 5.2. and table 5.2.). This study was part of a European collaborative project on spore forming bacteria (abbreviated as RASP), involving research institutes and food companies throughout Europe (Nauta 2000a, available via www.rivm.nl). It was found that consumers may be exposed to *B. cereus* by consumption of the products. The level of exposure is highly influenced by the consumer behaviour. With the present knowledge (which is, among others, characterized by the lack of dose response information), it was not possible to quantify the risk, or to draw any 'certain' conclusions on the risk of the product.

At the moment there is no dose-response relationship available for *B. cereus*. Based on epidemiological studies, a concentration of 10^5 cfu/g is generally considered as a critical value. As an estimate, at the moment the consumer takes the product from the refrigerator there may be a probability up to 6.5% of dealing with a pack that contains more than 10^5 cfu/g, if contaminated with a psychrotrophic *B. cereus* strain. This is, however, an uncertain estimate and its implications for public health are obscure. Nevertheless, some promising risk mitigation strategies can be identified, which will effectively lower the exposure to *B. cereus*. The most obvious ones are decontamination of some ingredients added during the production of the product and improved temperature control of consumer refrigerators. Controlling for food safety at the end of the industrial process by taking random samples there appears to be a bad predictor for food safety risk for the consumer.

Figure 5.2.(below). Food pathway of broccoli puree. The pathway is split up into seventeen modelling steps. The illustration shows how the units are formed by partitioning and mixing. As much as possible, a basic process is assigned to each of the steps.

Basic process



step	*process*	unit	time (h)	temp (°C)	description
1	*start*	broc. heads (400 g each)	0		As input the model requires the prevalence of B. cereus contaminated broccoli heads and the level of contamination on those
2	*growth*	...	196.0	5.7	Growth may occur at storage
3	*removal*	...	196.0	5.7	Ugly and dirty heads are removed
4	*growth*	...	224.0	7.0	Growth may occur at storage
5	*mixing, inact.*	280 kg batch	224.2	85.0	Heads are mixed into a batch. Temperature may lead to inactivation.
6	*growth*	...	224.3	20.0	Growth may occur at storage/cooling
7	*inact.*	...	225.1	97.5	Cooking will lead to inactivation
8	*inact.*	...	225.3	70.0	Temperature at blending may lead to inactivation.
9	*mixing, inact.*	325 kg batch	225.8	65.0	Ingredients are added to the vegetables. As extra input the model requires the prevalence of B. cereus contaminated ingredients and their level of contamination. Temperature may lead to inactivation.
10	*partit.*	380 g packs	226.3	58.3	Packages are filled with puree from the batch
11	*inact.*	...	232.4	70.0	Pasteurisation will lead to inactivation
12	*inact, growth.*	...	232.7	drops	During cooling conditions may be suitable for inactivation and growth
13	*growth*	...	284.7	4.0	Growth may occur at storage in the factory
14	*growth*	...	323.7	4.67	Growth may occur during transport from factory to retail
15	*growth*	...	472.2	4	Growth may occur at storage in retail
16	*growth*	...	472.9	11.5	Growth may occur during transport from retail to the home
17	*growth*	...	578.1	6.64	Growth may occur at storage in the home

Table 5.2. The food pathway split up in steps as illustrated in figure 5.2. For each step the basic process (inact. = inactivation), the unit size, the processing time and the process temperature are given as mean values. Time values are cumulative. Note that the 'growth' does not necessarily imply multiplication of cells; it implies that a growth model is used that includes the germination

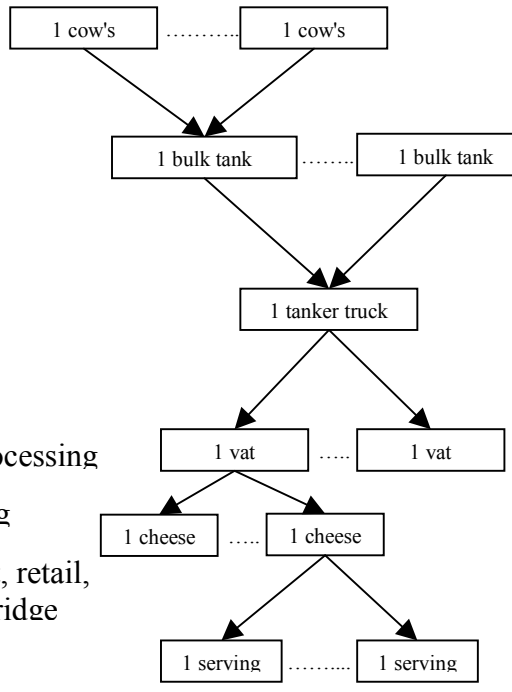
and lag time that precedes growth and that the probability of growth is assessed. (The same holds for inactivation.) See Nauta 2001a for details.

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

It was concluded that using MPRM is a promising approach, but that an improved interaction between risk assessment and microbiology is necessary.

5.3. Soft cheese made from raw milk

Quantification of the risk for public health resulting from consumption of soft cheese made from raw milk was attempted within the frame of the Codex Alimentarius Commission 1995 approach to quantitative risk assessment using Monte Carlo simulation software (Bemrah *et al*., 1998).



	Process steps	Units	Basic processes taken into account, *not taken into account*
Step 1	Milking	1 cow's ….. 1 cow's	**Contamination**
Step 2	Filling the tank	1 bulk tank …… 1 bulk tank	**Mixing, contamination**
Step	Storag		**Growth**
Step 4	Collection	1 tanker truck	**Mixing**
Step	Transport		**Growth**
Step 6	Storage	1 vat ….. 1 vat	**Growth**
Step 7	Before cheese processing		**Partitioning**, *removal*
Step 8	Cheese processing	1 cheese ….. 1 cheese	**Partitioning**, *decline, growth*
Step 9	Storage, transport, retail, transport, home fridge		*Removal, decline, growth, cross-*
Step 10	Consumption	1 serving ……... 1 serving	**Partitioning**, *decline, cross-contamination*

Figure 5.3. The food pathway of soft cheese made from raw milk split up into 10 steps as illustrated in the table. For each process step, the unit and the basic process (cross-cont. = cross-contamination) involved are described.

Quantitative data could only be found for *Listeria monocytogenes*. The complete process of cheese making was modelled, from milking to consumption. Using data published on the different sources of milk contamination (environment and mastitis) and bacterial growth, distributions were assumed for parameters of the model. Equations of Farber, J.M., Ross, W.H., Harwing, J. (1996) for general and at-risk populations were used to link the ingested dose of L. monocytogenes to the occurrence of listeriosis. The probability of milk contamination was estimated to be 67% with concentration ranging from 0 to 33 CFU ml-1. The percentage of cheese with a predicted concentration of L. monocytogenes greater than 100 CFU g-1 was low (1.4%). The probability of consuming a contaminated cheese serving was 65.3%. Individual annual cumulative risk of listeriosis, in a population each consuming 50 servings of 31 g, ranged from 1.97 x 10(-9) to 6.4 x 10(-8) in a low-risk sub-population and 1.04 10(-6) to 7.19 10(-5) in a high-risk sub-population. The average number of expected cases of listeriosis per year was 57 for a high-risk sub-population and one for a low-risk healthy sub-population. When the frequency of environmental milk contamination was reduced in the model and *L. monocytogenes* mastitis was eliminated, the expected incidence of

Step	***process***	**unit**	**description**	**Input data**
1	*start*	1 cow's milk	Milking	Prevalence and concentration of infected cow's milk
2	*mixing, cross-cont*	1 bulk tank	Filling the tank	Herd size; Prevalence and concentration of cross-contaminated bulk tanks
3	*growth*	...	Storage in the bulk tank	Time-temperature, growth parameters
4	*mixing*	1 tanker truck	Collection	Number of farms per collection
5	*growth*	…	Transport	Time-temperature, growth parameters
6	*growth*	...	Storage	Time-temperature, growth parameters
7	*partitioning*	1 vat	Partitioning into vats before cheese processing	Volume of one vat
8	*partitioning*	1 cheese	Processing: growth or inactivation during processing are not taken into account by Bemrah *et al*. (1998)	Volume of milk used for one cheese
9	-	…	Storage, transport, retail, transport, home fridge: not taken into account by Bemrah *et al*. (1998)	-
10	*partitioning*	1 serving	Partitioning into servings	Mass of one serving

Table 5.3. The food pathway of soft cheese made from raw milk split up in steps as illustrated in the figure. For each step, the basic process (cross-cont. = cross-contamination) taken into account by Bemrah et al. (1998), the unit, the description of the process step, and the necessary input data are indicated.

listeriosis decreased substantially; the average number of expected cases was reduced by a factor of 5. Thus the usefulness of simulation to demonstrate the efficiency of various management options could be demonstrated, even if results should be interpreted with care (as many assumptions had to be made on data and their distributions.

5.4. Pathways to be included in risk assessment of Campylobacter in chickens.

A quantitative risk assessment on *Campylobacter jejuni* in whole, chilled or frozen chicken products in Denmark has been developed. To quantify the health risks attributed to *Campylobacter* contaminated chickens, two models were developed: one describing the transfer and spread of *Campylobacter* through a chicken slaughterhouse and another dealing with the transfer and spread of *Campylobacter* during food handling in private kitchens. Uncertainty and variability linked to the model parameters were included.

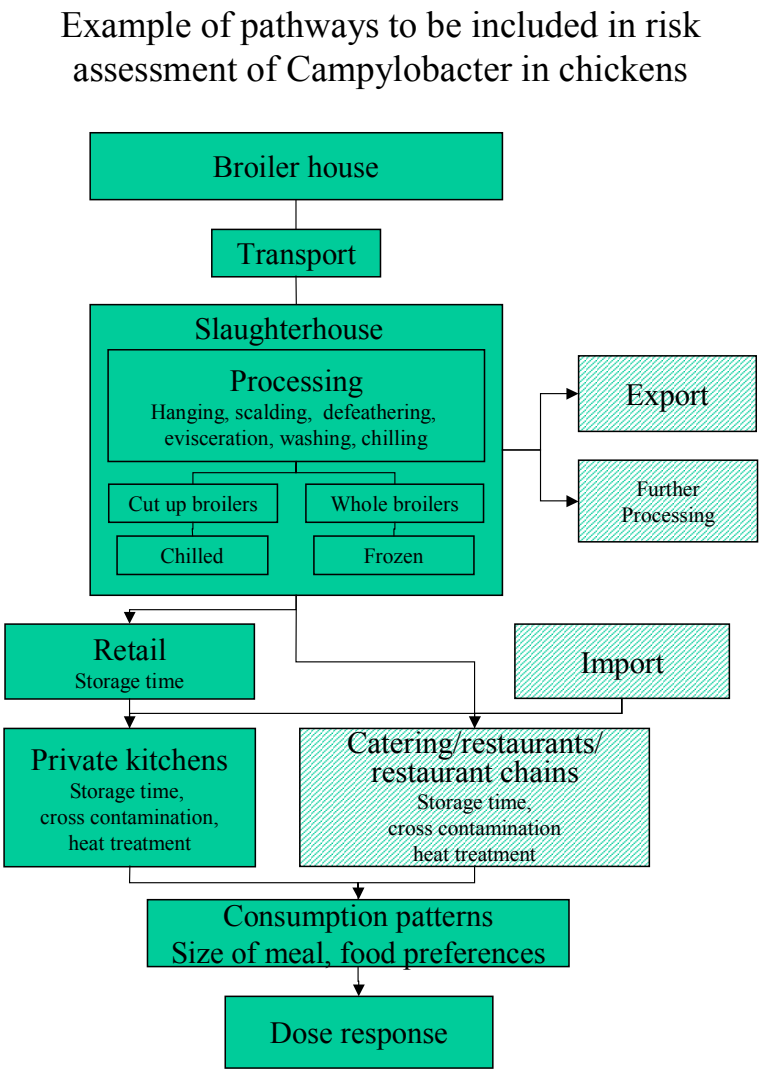
In the slaughterhouse part, changes in prevalence and load on contaminated carcasses throughout the different processing steps were modelled using a simple linear modelling approach. Cross-contamination between negative and positive flocks was modelled as an overall process assuming an exponential reduction in the *Campylobacter* load as function of the number negative broilers slaughtered after the last positive broiler.

The route of transfer modelled in the consumer part was the cross-contamination of prepared chicken and salad via unwashed cutting boards. Consumers were split into those preparing the food and those ingesting a serving, allowing the inclusion of the variable hygiene levels of the persons preparing meals. With respect to persons ingesting a serving, the size of meal and the number of people ingesting salad with the chicken was included.

By combining the two models, the effect of different mitigation strategies on the probability of exposure and illness could be analysed. In particular strategies, which reduced the *Campylobacter* load on chickens, seemed to have significant impact on the number of human cases. Cross-contamination from positive to negative flocks had almost no effect, which may indicate that logistic slaughter has a minor influence on the risk. Finally, the simulations showed that people in the age of 18-29 years had the highest risk of illness, a result, which is in good agreement with current observations.

In Figure 5.4. a schematic overview of the important steps to be considered in an exposure assessment of Campylobacter infected broiler-chickens is given. The grey shaded boxes (modules) represent those parts that were included in previous quantitative risk assessments (Christensen *et al.* 2000., Hartnett *et al.* 2001, Fazil *et al.* 1999) Although not modelled in exactly the same way as described by the Modular Process Risk Model (MPRM) methodology, a number of basic processes was included in each of the modules modelled. The basic processes were: At the slaughterhouse, cross contamination, inactivation (e.g. by scalding) and removal (e.g. by the scald, washing and chiller water flow), at the retail level; inactivation (freezing), at private kitchens; cross contamination, inactivation (e.g. by drying out or heat treatment) and removal (e.g. by washing utensil and cutting board). Note that growth of Campylobacter was not considered to occur in any of the processing steps, because Campylobacter do not to grow at temperatures below 30°C. The

private kitchen module is discussed in more details in section 4.3.



5.5 Cold smoking of fish as a generic fish HACCP example.

As described in the previous sections, the food pathway(s) of interest invariably have to be split up into smaller steps, or modules, in order to be able to model and estimate the exposure. If industrial or other production processes are part of the pathway of interest, the availability of HACCP-plans can be helpful in the development of the exposure assessment. Below is an example, which illustrates how a HACCP-plan describing the production of cold-smoked fish is used as a basis for identifying the crucial steps and the six basic processes in the MPRM approach that may need to be taken into consideration in the exposure assessment. The HACCP plan used in this example is generic and a greater level of detail may be desired.

Identification of basic processes (Nauta 2001) in generic description of process for cold-smoking of fish

Step	Description	Unit	Basic Processes
1. Receiving	Refrigerated or caught fresh or frozen	Whole fish/or fillets	Partioning (filleting)
	1. Clean appropriately or Thaw		Inactivation/ removal
	2. Wash in potable water		Removal/inactivation
2. Storage (Fresh/frozen)		Whole fish or fillets	Inactivation /growth
3. Thawing, washing, and rinsing		Whole fish /or fillets	Inactivation/removal
4. Butchering and evisceration		Whole fish /or fillets	Cross-contamination /removal/
5. Sorting, sizing, salting		Separate fish/fillets uniform size	
		Whole fish /or fillets	Partitioning/mixing/cross-cont/remov.
		Salting	Inactivation/cross-cont.
6. Drying, smoking	Removing fish from brine	Whole fish /or fillets	Inactivation
	Drain and/or rinse with fresh water		Inactivation/removal
	Drying		(Growth)/inactivation/cross-cont
	Smoking		Inactivation
7. Cooling		Wholes fish /or fillets	Inactivation/(growth)/cross-cont.
8. Slicing and cutting		Fillets	Partitioning/cross-cont.
9. Packaging		Vacuum /MA package	Mixing/cross-cont.
10. Storage and distribution		Package	Growth/Inactivation

Note this outline may not deal with the primary production in this case e.g., catching of fish, bleeding, degutting, and storage. In this regard, the primary stage is like a black-box.

Putative steps at the primary stage

Step	**Description**	**Unit**	**Basic Processes**
1. Catching of fish	Bringing the fish up from the sea	Whole fish	Cross-contamination and mixing
2. Bleeding	The caught fish is bleed	Whole fish	Cross-contamination
3. Degutting	The intestines / organs are removed	Whole fish	Cross-contamination
4. Storage	Degutted fish stored for processing	Whole fish	Growth, inactivation

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6. Sources of data

6.1 Introduction

There is usually a large gap between the data desired by the modeller and those available in the literature or in publicly available databases. Data may not have been identified, they may not be fully representative of the system to be modelled, either because they were collected in a different system or because they do not cover all model requirements. Data may only be available in aggregated form.

To overcome these problems, several strategies may be followed. These include:

- Obtain raw data from investigators;
- Dedicated efforts to retrieve unpublished information. Attention must be given to the quality assurance of the process by which these data have been collected;
- Use available data with additional assumptions on their relevance to the system that is modelled;
- Use expert opinion, by a formalised process of data collection and analysis;
- Perform additional studies to collect missing data.

The data needs for QMRA are considerable, and it is sometimes suggested that these may be prohibitive to carrying out risk assessments. This position, however, needs to be considered with care. If data are insufficient for risk modelling, they may likewise be insufficient for well-funded risk management decisions and QMRA is a useful tool to detect significant data gaps. Large sums of money are being spent each year to analyse food products and their ingredients for a wide variety of hazardous agents. These measurements are carried out as part of statutory requirements, for quality control purposes, for research projects etc. Measurements may be part of long-term monitoring and surveillance programmes or may be set up as short-term projects. The priorities for these measurements come from very different perspectives and purposes. Within the total budget spent on measuring the quality of the European food supply, the needs for reliable exposure assessments are relatively modest. Hence, the problem is essentially one of communication and prioritisation. Risk assessors should carefully communicate their data needs to both risk managers and scientists involved in observational or experimental studies, and the latter should promote incorporation of the necessary data collection efforts within current budgets. This may require reprioritisation, but given the direct support that risk assessment can give to risk management activities, this may even be beneficial for the process of decision making about research funding. Finally, it must be emphasised that pathogens in food do cause illness every day, in contrast to a wide range of regulated chemicals that are present in amounts below the threshold of concern. Yet vast amounts of data are available on the chemical contamination of the food supply, and in contrast very few data on pathogens themselves are available. Likewise, there is a large effort in monitoring for indicator organisms in quality control in comparison to limited testing for pathogens. New priorities, based on direct relevance to health risk assessment may help to provide the required data for risk assessment within existing budgets.

6.2 Data for exposure assessment

This chapter includes information from the FAO/WHO Guidelines on Exposure Assessment of Microbiological Hazards in Food and Water, Draft 11 December 2001 in which more details are given.

The aim of the modelling of each of the basic processes is to describe the change in prevalence and number of microorganisms per (contaminated) unit for each processing step, and this preferably in quantitative terms. For this, data will be needed on environmental conditions (e.g., temperature, pH) and (handling) practices (e.g., duration of transport, storage) at the various processing steps. To validate the model, data are needed on N, unit size and P at the start and end of all steps.

Also data on amount and frequency of food intake in the given population or subpopulation is needed.

Basically two categories of data are necessary for the development of an exposure assessment: firstly, data that describe the biological and physical processes as well as the human factors involved and, secondly, numerical data that allow quantitative estimates of exposure to be calculated. The extent to which numerical data is required will vary from one assessment to another, depending on the purpose and scope defined for an exposure assessment and the modeling approach and details chosen.

The types of data possibly used in an exposure assessment comprise data on the food product, the food chain, the microorganism and the consumer.

The food product.
When performing an exposure assessment of a given microorganism-food combination, the food product concerned should be fully described. A complete description of the food include a list of ingredients that the product is composed of as well as information on the ability of the product to support growth/survival, defined in terms of e.g. pH, salt, types of packing etc. Also the storage time and temperature as well as information of use-by-dates are necessary information that should be used to predict possibilities for growth/survival. Information on country/region of origin and type of producer and processing plant is also important information which unfortunately quite often is missing when foods are surveyed for contamination. In addition information on sampling plans should be provided since it should be clear whether samples are independent samples or analytical replicates.

The food chain.
The food chain consists of all stages from the (primary) production to the home of the consumer or to the restaurant/food service establishment. If the whole food chain is to be modelled then a description of all stages is required.

Although this may seem easy at first hand, experience has shown that a description in quantitative terms (number of animals and their destination or their origin (national, import), number and weight of carcasses and their destination or their origin, etc) is not easily obtained. Moreover, when the model is to be used also to gain insight into risk factors and risk reduction scenario's, data on alternative food pathways and/or steps will be needed. In case the risk may vary between different production systems or is the subject of the study (e.g., intensive (industrial) *versus* extensive (ecological) systems), data from various totally different production chains will be needed.

The production of a food commodity is a complex process that may involve several stages where some or all the basic processes (growth, inactivation, cross-contamination, mixing, partitioning, removal) may occur. Typical data requirements will include the extent to which (in quantitative

terms) the different events occur. With respect to food handling practices in the homes, restaurants and food service establishments, adequate descriptions of the processes are only available occasionally (see also sec. private kitchens).

Throughout the food chain, many control options are available to minimise the risk of microbiological contamination of the final food product. These may be incorporated in Hazard Analysis Critical Control Points (HACCP) plans that are specific for each product and manufacturing site and, thus, may vary substantially between manufacturers. Data that describes both the methods of control, and the extent to which these vary, may be relevant for specific exposure assessments.

Data used in the exposure assessment should be collected to represent as closely as possible the conditions and practices prevailing in the different stages of the food chain studied. Not in all instances this will be possible, and alternative data need to be used. It is important to clearly describe the rationale for selecting the alternative (surrogate) data and to what extend they do or do not describe the prevailing conditions.

The microorganism.
In a specific exposure assessment, the number N, the unit size, and the prevalence P, of the microorganism concerned in the beginning and end of each relevant basic process is needed in order to validate the models used.

When obtaining data of prevalence and numbers of a specific microorganism the sensitivity and specificity of the analytical methods should be known. The sensitivity is the capability of a method to correctly identify a positive sample. A method of too low a sensitivity will result in the occurrence of false negatives. The specificity is the capability of a method to correctly identify a sample as negative. Too low a specificity may result in the occurrence of (too many) false positives. Changing methodologies, particularly improvements in the selective agars, need to be evaluated. If a method has a known low sensitivity this could be adjusted for when calculating the prevalence. The problem is much bigger if we don't have information on the sensitivity.

After having collected data to describe the starting levels of exposure, then information related to changes in the likelihood and level of contamination throughout the food chain is necessary. Depending on the particular stage, there may be sufficient information that describes actual changes. Alternatively, it may be necessary to *predict* changes in likelihood and levels and thus data to enable such predictions will be required.

Prediction of changes in the level of contamination on the product throughout the food chain will be based, primarily, on predictive microbiology and thus on growth and survival models. Information on growth and survival at different levels of temperature, pH, oxygen and salt content may be relevant as are published or calculated growth rates and D-values. Also the presence of a competitive microflora may relevant since it has been shown that proliferation (rate and/or extend) of the spoilage microflora of a product influences the behaviour of the pathogen concerned (i.e. *Listeria* on smoked salmon).

In planning and executing an exposure assessment, the variability in infectiveness or toxicity of pathogens should be considered such that representative data are collected. Obviously there is

variability between genera or species, but even within a species only certain sub-types will be relevant in terms of pathogenesis.

The physiological and physical state of the microorganism in the food remains a relatively unexplored area. Stress, injury and recovery also affect the initiation of growth. Most frequently studies use stationary phase cells grown in a nutrient-rich broth at favourable temperatures and the predicted lag phase duration represents those conditions; cells that contaminate a food may not be in the same physiological state as these cells.

Generally we are only interested in modelling the exposure to microorganisms that are infectious or toxic and it is therefore necessary to consider whether our methods of detection are capable of that. In that respect a method might be too sensitive (i.e. a PCR-based method may detect DNA from "dead" non-infectious microorganism) or not sensitive enough (i.e. not able to detect stressed "still infectious" microorganisms).

In microbiology, the terms "culturable" and "viable" are usually equated (Kell et al., 1998); for instance the so-called "total counts" are invariably expressed as colony forming units, which by definition represent the culturable fraction of a total population.

In recent years, starting from studies in environmental microbiology, it has become clear that microbial cells can exist in both viable and *culturable* or viable and *non-culturable* (VNC) state. In particular, the latter has been demonstrated for a number of human pathogens (Besnard et al. 2000; Tholozan et al. 1999) including *Escherichia coli, Salmonella enteritidis, Vibrio cholerae, Legionella pneumophyla, Campylobacter jejuni* and several bacteria used in agriculture as seed/soil inoculants (Toffanin et al. 2000). The VNC state can be assumed to be a transitory condition of bacteria they undertake to overcome adverse environmental situations such as gaseous oxygen limitation, pH and temperature variations, salt concentration, and nutrient deficiencies. The VNC of *Ralstonia solanacearum* and *Pseudomonas fluorescens* cells can persist for a long time (Grey and Steck, 2001; Lowder et al. 2000), although they do not show enhanced persistance (Mascher et al. 2000), unless conditions facilitating the retrotransition occur.

Although some of the mechanisms governing the resuscitation of VNC cells begin to be unravelled (Toffanin et al. 2000), the field remains at large to be elucidated. Therefore, for an appropriate and comprehensive risk assessment procedure it appears that the assumption on the coupling between microbial growth and metabolism should be justified for each microorganism and detection methods and data collection adjusted accordingly.

The consumer

The final phase of the exposure assessment is directed towards the consumer. Two types of information are used in characterizing dietary exposure to pathogens: information on consumer behaviour and data on food consumption patterns (See also chapter 4.3 and 4.4 regarding handling in private households and consumption patterns).

Relatively little information exists on the food handling practices in the home that may affect the safety of foods. Food handling practices vary by geographic region or even within the same country based, for example, on ethnicity, gender and education. Consumer storage times, extent of cross contamination, cooking times and temperatures, hot holding temperatures and times, and other data are not generally available. Likewise, little information is available about food handling practices

by restaurant and food service operations including street food, which accounts for an increasingly greater proportion of meals in many countries.

Regarding food consumption, in general terms it is necessary to know the amount of food consumed and the frequency with which the food is consumed. The specific expression or characterization of food consumption patterns used in the microbiological risk assessment depends upon the question to be answered by the assessment as well as the food consumption data that are available to the risk assessor. Food consumption patterns will likely differ based on population demographics (age, gender, ethnicity, health status, socio-economic group) and seasonal and regional (both national and international) differences in food availability. For microbiological risk assessments, consideration of food consumption patterns for sensitive subpopulations (e.g., young children, pregnant women, the elderly, and the immunodeficient) and high-risk consumer behaviour (e.g., consuming unpasteurized dairy products or undercooked meat products) are particularly important. Information that enables the estimation of variability in serving size will also be important.

The two types of food consumption data most frequently used for characterizing food consumption patterns for microbiological risk assessments are food production statistics and food consumption surveys. Other sources of information such as retail food sales or purchase data may be useful in filling data gaps in either food production or food consumption survey data.

6.3 Dealing with data gaps

Frequently the risk assessor and managers have to deal with missing, incomplete, incomparable information sources, biased data or the results are not representative. While, these situations are annoying it is possible to deal with them with using several tools. Hence, they indicate that more work is needed rather than the analysis cannot be done. Rather one should try to deal with them in a structured and transparent way. The data gaps should be clearly communicated to risk managers. When preparing a risk profile one should identify the data gaps and outline strategies for how they should be dealt with.

It might be useful to deal with data gaps in two ways to overcome data limitations and improving data collection.

Model simplification.
A model will always be a simplification of real life. If modeling the food chain it may not be necessary to model all steps in the food chain. If the purpose is to look at the risk for the consumer, it may not be necessary to model all intermediate steps or applying black-box models for these intermediate steps. The model will still give useful results at the point of interest i.e., risk from consumption of hamburgers while it may not describe what is the critical factors when mincing meat or during slaughter. This depends on the scope and purpose of the risk assessment.

The advantage of this approach is that simple models are easier to understand, and sometimes more focused on the question at hand, while the disadvantage is that one might assume away reality losing critical information.

Predictive microbiology and surrogate data
If there is missing data at the point of consumption some of the data gaps might be filled with results from growth models. For example while the prevalence and/or quantity of a pathogen in a

foodstuff might be known at the point of production, the similar figures are not available for the point of consumption. In these circumstances predictive microbiology might be helpful (see chapter 3 for an extensive discussion).

Surrogate data could be derived from comparative studies. That is one approximation of the survival of Escherichia coli O157 on pasture could be the survival of coliforms on pasture. If one has studies from other countries for E. coli O157 and survival time on pasture they could extrapolated to the current analysis. However one should be careful to note the impact of these data. Another example is the extrapolation of animal research to humans when trying to establish the dose response relationship. The use of surrogate data can be very helpful to get guesstimates or reasonable ranges to use in an exposure assessment. However, the use of such data should be explicit and transparent to the end users.

Expert opinion
When data is not at all available, or one is working on exceedingly rare events expert opinion might be the only method available. It is the opinion of the Working group that the use of expert opinion should not be limited to assessments where limited information is available. Expert opinion should also be used to complement other data sources. When there are large deviations between the results obtained from different sources it appears that one has to assume a great deal of uncertainty and proceed with caution. Gallagher, 2002, reported the findings from a workshop in UK September 2001 for eliciting the expert opinion on the FMD risk for countries in the EU. The opinion was that it was approximately 2% annual risk of FMD outbreaks in the low risk countries in the EU (British Isles and Nordic countries). Moreover, the most likely scenario for introduction was the illegal or tourist and immigrant imports of animal products such as meats. Based on this is appears that expert opinion is useful for building likely scenarios rather than eliciting precise estimates to be used for calculating exposures.

Data collection
Exposure assessments require information on the prevalence and concentration of the pathogens in the foodstuffs in questions. One issue is that the data is biased that is fraught with systematic errors. For example, if one observes an increase in pathogen prevalence in a foodstuff it could be that one has a true increase in prevalence or an apparent increase in prevalence due to larger sample sizes or improved methodologies. Another issue is that data obtained from different sources. One structured way of dealing with this is the use of meta analyses. For a guide to this approach see Greenland, S, 1998. Meta-analysis, chapter 32, pages 643-673, in Eds Greenland, S, Rothman, KJ. Modern Epidemiology, Lippincott-Raven, Philadelphia, USA). If one is attempting to aggregate data from several data sources one should avoid a mechanical adding up of numbers but rather look for similar trends in the different studies. In general the principles for the good surveillance or monitoring do apply with clearly defined research questions, reference populations, study populations and sampling frames. One guide for such sampling is in the SSC opinion on requirements for statistically authoritative BSE/TSE surveys of November 29-30, 2001. Another good reference text when planning surveys is Thrusfield (1996) in (Thrusfield M., 2001. Veterinary Epidemiology 2nd edition, chapter 13, Blackwell Science, Oxford, UK).

The forthcoming FAO/WHO Guidelines on Exposure Assessment of Microbiological Hazards in Food and Water (Draft 11 December 2001) will also be useful in this respect.

6.4 Discussion and conclusions

The aim of the modelling of each of the basic processes is to describe the change in prevalence and number of microorganisms per (contaminated) unit for each processing step, and this preferably in quantitative terms. For this, data will be needed on environmental conditions (e.g., temperature, pH) and (handling) practices (e.g., duration of transport, storage) at the various processing steps. To validate the model, data are needed on N, unit size and P at the start and end of all steps.

Another category of data that is needed concerns the description of the food pathway. Although this may seem easy at first hand, experience shows that a description in quantitative terms (number of animals and their destination or their origin, number and weight of carcasses and their destination or their origin, etc) is not easily obtained. Moreover, when the model is to be used also to gain insight into risk factors and risk reduction scenario's, data on alternative food pathways and/or steps will be needed. In case risk may vary between different production systems or is subject of study (e.g., intensive (industrial) *versus* extensive (ecological) systems) data from various totally different production chains will be needed.

A third category of data is associated with the definition of the population for which the exposure assessment is done. Exposure assessment should provide an estimate with associated uncertainty of the (variability in) occurrence and level of the pathogen in a specified portion of a certain food at the time of consumption in a specified population. It should therefore identify the food consumption frequencies in a certain time period and the weights consumed in a given population or subpopulation and should combine the information to estimate the population exposure to the pathogen under study through the specified food commodity. Therefore data on amount and frequency of food intake in the given population or subpopulation is needed.

Note should be taken of the dependency of the secondary data that is data collected for other purposes than the risk assessment. Currently the collection of data for food borne zoonoses is revised within the Community and a pertinent question would be that if the collection was pulled by the needs for QMRA would the priorities be different than what is collected today. One priority could be the comparability of data e.g that sampling is done on the same amount of foodstuff (1 gram, 25 grams).

Frequently the risk assessor and managers have to deal with missing, incomplete, incomparable information sources, biased data or the results are not representative. While, these situations are annoying it is possible to deal with them in two ways to overcome data limitations and improving data collection. These are model simplifications and predictive microbiology including use of surrogate data handled through an expert opinion. An expert opinion will complement other data sources in particular in building realistic scenarios. In addition the methodology of meta-analyses can be used for collating and analyzing data from different sources.

Nevertheless it is crucial that risk assessors carefully communicate their data needs to both risk managers and scientists involved in observational or experimental studies, and that the latter promote incorporation of the necessary data collection efforts within current budgets.

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7. Development and presentation of exposure estimates

7.1 Choosing a modelling strategy

As indicated in chapter 3, risk assessment models can be classified according to different principles. It was recommended that whatever types of models are chosen, they should have a sound biological basis. This chapter gives some general guidance on choosing between different modelling approaches, based on the purpose of the analysis and the available data. We will discuss the choice between mechanistic and statistical models, how to handle the effects of chance, how to incorporate time-dependent effects and how to solve the mathematical equations. The process of model development is an iterative process, wherein initial choices are refined and modified as the insight in the underlying process grows and the availability of data is explored in greater depth. 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. However, this may be difficult in practice, since the choice of model may be very dependent on the data that is available (Nauta et al, 2001).

Mechanistic and empirical models
A fundamental choice is between mechanistic (explanatory) models and empirical (statistical, associative) models. In general, mechanistic models capture the details of the process under consideration in greater detail than empirical models. The latter provide a mathematical description of the relation between the input and the output of a particular process and do not attempt to describe the processes that underlie the input-output relationship. They have been referred to as black-box models in chapter 3. Note that also when using empirical models it is important that the functional form of the model has biological plausibility and that the model parameters have a biological interpretation. Usually, there are many different model forms that can be used to describe a given dataset and the choice between the different options should not be guided by the data alone. Empirical models are efficient because they are easier to build and implement than mechanistic models. There may be several reasons for not using empirical models and to invest resources in the development of mechanistic models. One important reason is the need to estimate the effect of specified interventions in the food chain. In general, stages in the food pathway where interventions are to be evaluated need to be modelled in more detail than other stages. Another reason may be that a particular stage is highly critical in terms of introducing or reducing the level of hazards, and a simple empirical model may not be able to accurately describe the effects of that stage. In general, it is not recommended to use empirical models to extrapolate beyond the domain of observed data unless the functional form of the model has a well-defined biological basis. For example, polynomial secondary growth models (see Table 3.2) can be very useful because they describe large experimental datasets, but their behaviour outside the observed range can be quite erratic. Square root models such as the Gamma model have biologically interpretable parameters and are more robust with regard to extrapolation.

Dynamic and static models
Current risk assessment models generally are of a static nature, i.e. they do not explicitly consider the effect of time. The typical model considers the events that take place during a fixed period of time, such as one year and treats differences between or within time periods (e.g. seasonal variation) as variability. Embedded in these static models one may find dynamic modules such as for microbial growth or death. However, the output of these dynamic modules is usually of a static nature, e.g. the relative increase or decrease in microbial numbers.

Deterministic and stochastic models
Another important distinction is that between deterministic and stochastic models. In a deterministic model, the effects of chance are ignored and all parameters have a fixed value. The end result of a deterministic model is one point estimate. In a stochastic model, all events are considered as variable and are represented by probability distributions. It is also possible to express the uncertainty in the model parameters with a probability distribution. In exposure assessment, deterministic models are particularly useful in the first stages of a project, when the events that have a major impact on risk must be identified. Subsequently, stochastic models are usually constructed to fully account for variability and uncertainty in the most critical stages. An extensive exposure assessment may require the specification of over 100 variables. Only a few of these inputs probably will drive the assessment in terms of having a substantial impact on the magnitude or the ranges of predicted risks. These variables may be most suitable inputs for probabilistic treatment of uncertainty. It may be necessary to treat variability by a probabilistic method simply to get the model right and not depending on the effect on the output in the sense that you would evaluate uncertainty (Burmaster and Anderson, 1994). Similarly, 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. The variables and pathways left out of the probabilistic analysis and the reasons for doing so should be discussed in the assessment. A structured approach to developing an exposure assessment model and supported by an expert system was proposed by Van Gerwen *et al*.(2000). The so-called SIEFE system (Stepwise and Interactive Evaluation of Food safety by an Expert System) uses a 3-tiered approach to exposure assessment. First, risks are assessed broadly, using order of magnitude estimates. Characteristic numbers are used to quantitatively characterise microbial behaviour during the production process. These numbers help to highlight the major risk-determining phenomena, and to find negligible aspects. Second, the risk-determining phenomena are studied in more detail. Both general and/or specific models can be used for this and varying situations can be simulated to quantitatively describe the risk- determining phenomena. Third, even more detailed studies can be performed where necessary, for instance by using stochastic variables.

Analytical and numerical solutions
Only in the most simpel cases is it possible to solve such stochastic models analytically but in general numerical simulation methods are necessary. Monte Carlo simulation is a particularly useful tool for simulation of risk assessment models and is frequently applied. Other possibilities are Bayesian belief network (Barker *et al.*, 2000) and fuzzy methods (Ferson, 1996). There are many aspects that must be taken into account when constructing Monte Carlo simulation models. Details can be found in textbooks such as Morgan and Henrion (1990). Vose (2000) is a useful reference in the context of (microbiological) risk assessment, and also gives suggestions for further reading. Burmaster and Anderson (1994) give some useful tips for proper application of Monte Carlo techniques.

7.2 Variability and uncertainty

The probability distributions used in stochastic risk models may represent uncertainty as well as variability. In this context, uncertainty represents the lack of perfect knowledge of a parameter value, which can 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 further measurements. See Figure 7.1 for further details.

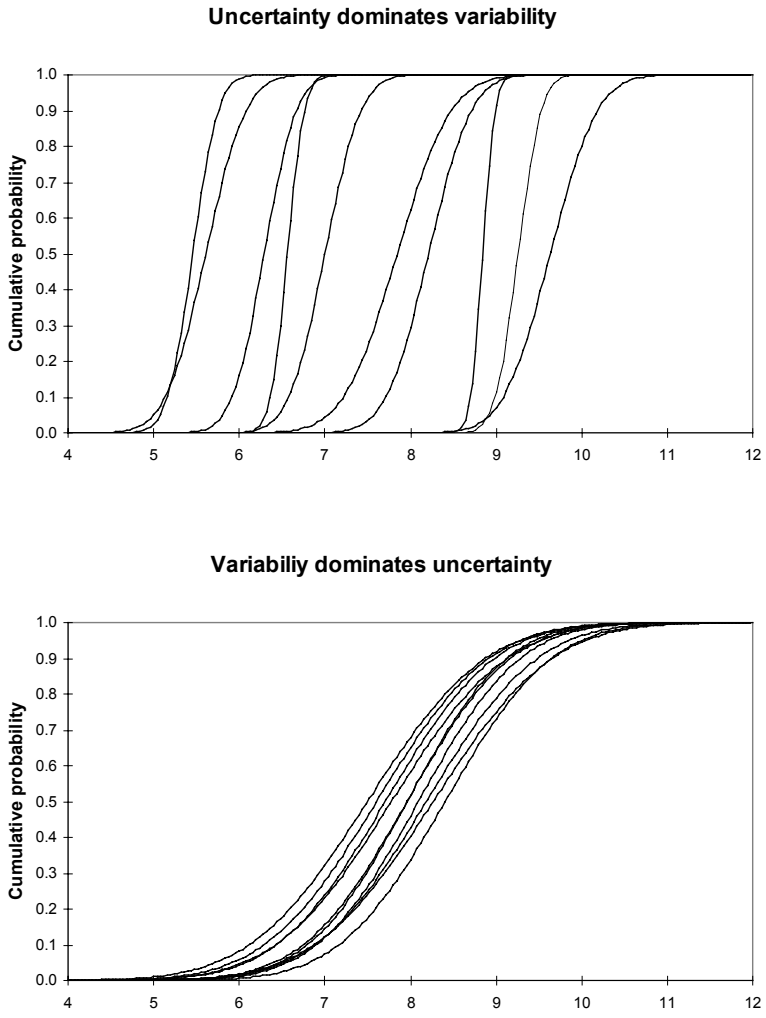


Fig. 7.1. An example to illustrate the difference between variability and uncertainty (after Vose, 2000). The upper panel represents a situation where the mean of a (normally distributed) parameter is highly uncertain, and varies between 5 and 10. The variability of the parameter is limited, with standard deviation between 0.1 and 0.5. The lower panel represents the opposite situation, in which the uncertainty of the mean of the parameter is small, and varies between 7.5 and 8.5. The parameter is highly variable, with standard deviation between 0.9 and 1.1. Both graphs show ten possible cumulative probability distribution functions of the unknown parameter.

Separation of variability and uncertainty in QMRA models (so-called second order models) has 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 for the inputs and the outputs is a goal of risk assessors, and such a separation allows decision-makers to understand how model outputs might improve if uncertainty is reduced. The core of a probabilistic model is the variability of the stochastic system, and once this variability model has been constructed the uncertainty of

parameters can be overlaid. There are essentially two methods for producing such 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 uncertainty distributions for uncertain parameters (Vose, 2000).

Considering the efforts involved, the value of making the separation must be carefully evaluated in each case. The scenario or event being modelled must be kept in mind to ensure that the model provides answers to the questions posed, that comparable data, which can be combined in the model, are used, and that an event that could physically occur in reality is described (Vose, 2000). For example, a model that directly multiplies a sample from the variability distribution of the number of pathogens per serving with the yearly total number of servings in a population is not a good representation of reality. This is because it includes the highly unlikely scenarios that all servings in a population contain the maximum or the minimum concentration of the hazard.

Types of uncertainty
Uncertainty, or our lack of knowledge, includes scenario uncertainty (descriptive errors, aggregation errors, errors in professional judgement, incomplete analysis), 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 parameter uncertainty (measurement errors, sampling errors, systematic errors) (Anonymous, 1997).

Although accounting for the important sources of uncertainty is a key objective in a Monte Carlo analysis it is not possible to characterise all the uncertainties associated with the model(s) and the data. However, the analyst need to attempt to identify the full range of types of uncertainties affecting the analysis and clearly state what set of uncertainties the analysis attempts to represent and what it does not (Anonymous, 1997).

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.

7.3 Sensitivity analysis

The assessment of the response of a model to the effects of different methods is often referred to as sensitivity analysis. There are different methods for sensitivity analysis, and each method gives insight in another aspect of this relation. Note that in many texts, the term sensitivity analysis is used for just one or a few of these methods.

Thus, there are several sources of uncertainty in the final results of a risk assessment model. Some of these are formally analysed by simulation. But any risk assessment study invariably involves many assumptions and interpretations of the available information. It is often not straightforward that a particular interpretation or choice input data or model structure is the optimum solution, and it rarely is the only solution. This constitutes a major additional source of uncertainty.

It is therefore important to find out which model parameters and structural assumptions (factors) of a model have the greatest effect on the model output (response). Factors can be qualitative or

quantitative. Apart from providing insight in the uncertainty of the model results, this sensitivity analysis is also critical in providing insight in the most critical data gaps, and thus to formulate key research recommendations.

There are different methods to assess the response of a model to the effects of different factors. Each method gives insight in another aspect of this relation. Some methods can be applied to the results of a single simulation run, and thus are computationally effective. Other methods, that usually give more information, require multiple simulation runs to be performed. With complex models, such as food risk models the required computational efforts may become prohibitive. Most methods are not routinely available in commercial risk analysis software and are not easily implemented in a spreadsheet environment. Thus, they are currently rarely applied in microbiological risk assessment. For more information, see Vose (2000), Law and Kelton (2000). Some important techniques for sensitivity analysis are described below:

- *Correlation analysis*. This analysis illustrates the degree to which the uncertainty in a model output is affected by the uncertainty in the model input. This is obtained by computing Spearman or rank order correlation coefficients between the model output and the input parameters. The results can be presented in tabular form, or as a tornado chart. This method of sensitivity analysis is implemented in popular risk analysis software such as @RISK and is therefore often reported in food risk assessments. It is important to note that the important factors identified by this method are not necessarily the parameters that have the largest effect on the model output. A parameter is identified as important mainly as a function of its uncertainty. Methods are also available to analyse second order simulation models, in which uncertainty and variability are separated.
- *Spider plots* are produced by setting each variable in the model at a fixed value, then subsequently varying an input variable by discrete steps and plotting the effects on the output as a function of the variation in the input. This process is repeated for each variable of interest and a combined graph is produced. The model is most sensitive for the parameters for which the plots have the steepest slopes. Several options are available to perform this method. One is to set each parameter at it's mean value and to vary the input parameters by fixed percentages, e.g. 10%, 20%, 50%, 200%, 500% and 1000% of the mean. This method is independent of the uncertainty of the input parameter, and produces insight in the effect of input parameters near the mean of the model output. An alternative method is to set all parameters at their median value, and to vary according to fixed percentiles of the cumulative distribution of the input parameters, e.g. 1-, 5-, 20-, 80-, 95-, and 99-percentiles. This method provides insight in the sensitivity of the model for the input parameters in their entire uncertainty interval.
- *Factorial designs*. If there are two or more factors in a model, it is of interest to know how each factor individually affects the output of a model and what is the interaction between these factors. Factorial designs are a method to evaluate these questions in an effective way. One example is the 2^k factorial design, in which each factor (qualitative or quantitative) is set at two extreme but realistic values, and simulation runs are 2^k performed with every possible combination of these factors. By systematically analysing the outputs of the model it is possible to study both main effects and interactions of parameters. This type of design is only applicable to models with relatively low numbers of factors. Less computer intensive designs are available, but in general the large number of factors in microbial risk models makes application of this technique difficult. A simple variant of this principle is to vary one factor or a logical combination of a small number of factors and repeat the simulation. Listing or graphing the changes in the model response provides a basic insight in the behaviour of the model. This is

however limited to the effect of one or a few factors in isolation and does not address the interaction between input parameters.

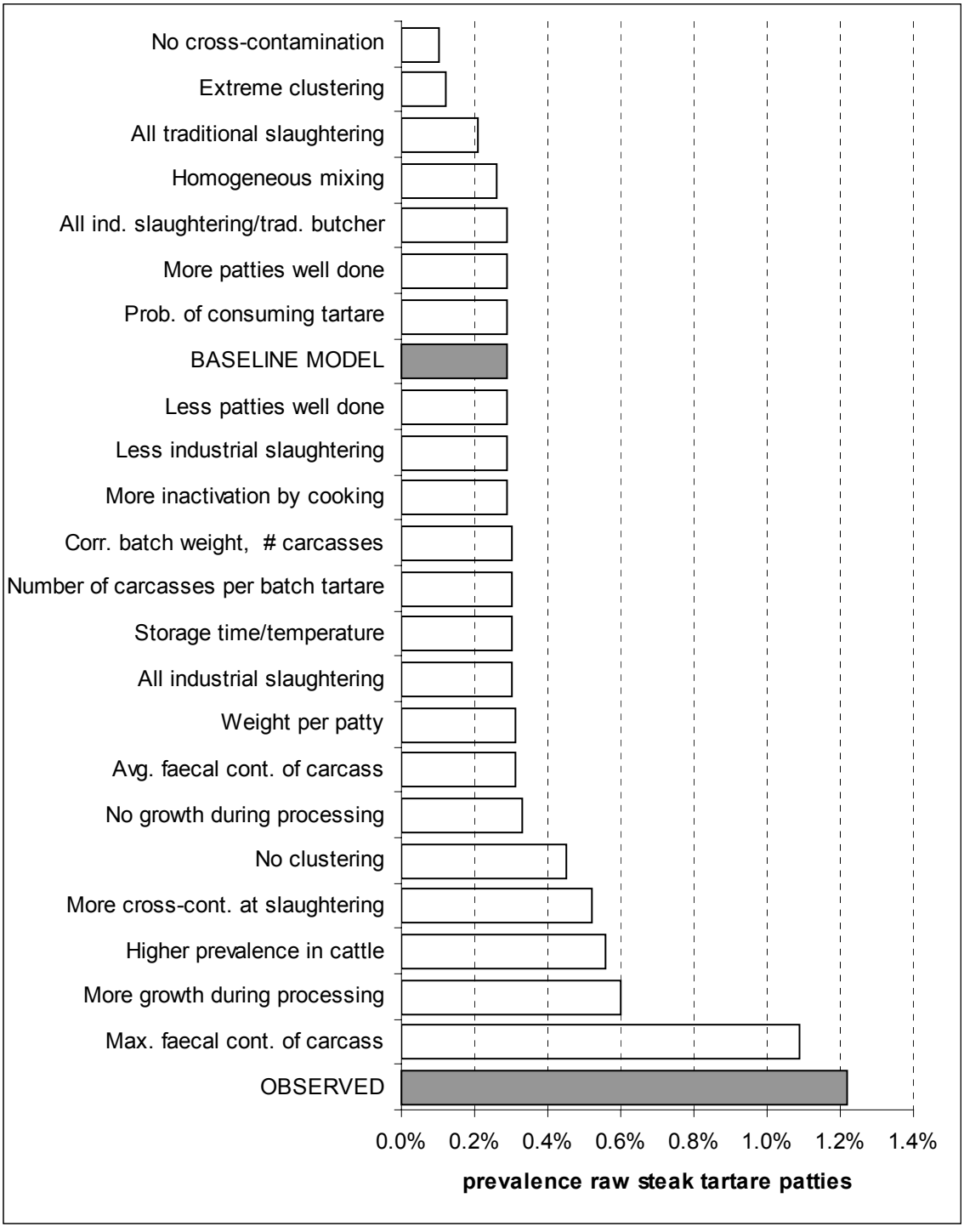
- *Gradient estimation*. For models with continuous parameters, it is of interest to know how the model response to small changes in the input parameters, i.e. to know the gradient (slope, partial derivative) of the response function with respect to the input parameters. Analytical solutions are only possible for simple models, but for most models the gradient must be estimated by simulation. This may quickly lead to complex and demanding calculations, and more efficient methods have been developed. One example is the perturbation method, where during the simulation the level of a parameter is slightly changed (perturbed) and the effect on the output is studied. A simple variant of this method sets each parameter at its mean or median value and then changes one parameter by a small percentage to evaluate the relative effect on the model output. An example is given in Fig. 7.2.

7.4 Reporting the results of exposure assessment

The results of an exposure assessment usually consist of a set of output values from a Monte Carlo simulation. It is important to carefully consider the presentation of such results. They must be meaningful to specialists who read and review the risk assessment, but also to readers who are less specialised in statistics and modelling. As a general starting point, the following presentation of results is recommended as a minimum:

- A listing of all input parameters and their distribution. It is advisable to also give some characteristic values such as mean or median and some percentiles (e.g. 5^{th} percentile and 95^{th} percentile). The added value of graphical representations (histograms, cumulative frequency plots) should be evaluated on a case-by-case basis.
- Mean, median, some percentiles and variance or standard deviation of all relevant output statistics.
- Typically, exposure models result in the prediction of the prevalence of contaminated food items, and the concentration of pathogens in a contaminated item. These outputs should be extensively characterised.
- It is usually instructive to also summarise the results of several intermediate steps in the food chain. Particular attention can be given to those steps where the risk manager plans interventions.
- A graphical presentation of all relevant output statistics. A histogram is easily understandable by most readers, but a cumulative frequency plot may be more informative for specialised readers.
- A graphical representation of the results of sensitivity analysis. It is advisable to also report these results in a tabular form. Apart from providing insight in the uncertainty of the model results, these scenario analyses are also critical in providing insight in the most critical data gaps, and thus to formulate key research recommendations.

Figure 7.2. Sensitivity analysis. Example of sensitivity analysis by variation of one or a few factors in the exposure model, based on Nauta *et al*. (2001). The baseline exposure model (BASELINE MODEL) estimated the frequency of contamination of raw steak tartare patties with Shiga-toxin producing *Escherichia coli* serotype O157 in the Netherlands. The sensitivity analysis compared the outcome of the baseline model with different alternative scenarios. For details the reader is referred to the original report. The sensitivity analysis indicated that in many scenarios the estimated prevalence was similar to the baseline prevalence. The results of all scenarios were also compared with independent observations (VALIDATION). Note that all model outcomes were actually lower than the observed prevalence.



7.5 Discussion and conclusions

The separation of variability and uncertainty in QMRA models has up to now rarely been made, a reflection of the fact that this can be a daunting task[1]. However, neglecting the difference between them may lead to improper risk estimates (Vose, 2000) and/or incomplete understanding of the results (Barker *et al*. 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 (Barker *et al*. 2000). The explicit separation of these two for the inputs and the outputs is a goal of risk assessors, and such a separation allows decision-makers to understand how model outputs might improve if uncertainty is reduced. The core of a probabilistic model is the variability of the stochastic system, and once this variability model has been constructed the uncertainty of parameters can be overlaid. There are essentially two methods for producing such 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 uncertainty distributions for uncertain parameters (Barker *et al*. 2000). Considering the efforts involved, the value of making the separation must be carefully evaluated in each case.

Uncertainty, or our lack of knowledge, includes scenario uncertainty (descriptive errors, aggregation errors, errors in professional judgement, incomplete analysis), 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 parameter uncertainty (measurement errors, sampling errors, systematic errors) (Morgan and Herion, 1990).

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[1] This assumes that variability and uncertainty have been defined earlier in the text!

8. Model validation and review[2]

8.1 Validation

Model validation can be defined as demonstrating the accuracy of the model for a specified use. Within this context, accuracy is the absence of systematic and random error, in metrology commonly known as trueness and precision. All models are, by their nature, incomplete representations of the system they are intended to model, but, in spite of this limitation, models can be useful. General information on working with mathematical models can be found in different theoretical and applied textbooks. Chapter 13 (Working with models) in Doucet and Sloep (1992), gives a very good introduction to model testing. These authors discriminate between model confirmation (shown to be worthy of our belief, plausible) and model verification (shown to be true). Chapter 14 (Constructing models) describes a structured approach to designing models and the pros and cons of various options. McCullagh and Nelder (1990) is a valuable resource on statistical modelling methods.

Chapter 3 of this report describes some general principles of applying mathematical models and underlines three principles for the modeller:

- All models are wrong, but some are more useful than others;
- Do not fall in love with one model to the exclusion of others;
- Thoroughly check the fit of a model to the data.

Law and Kelton (2000) give some useful perspectives on model validation:

- Conceptually, if a model is "valid", then it can be used to make decisions about the system;
- The ease or difficulty of the validation process depends on the complexity of the system, and on whether a version of the system currently exists;
- A simulation model of a complex system can only be an approximation of the system. There is no such thing as absolute model validity. The most valid model is not necessarily the most cost-effective.
- A simulation model must always be developed for a particular set of purposes.
- Validation is not something that should be attempted after the model has been developed, and if there is time and money remaining.

Four major aspects of model validation can be recognised (Dee, 1995):

- Conceptual validation
- Validation of algorithms
- Validation of software code
- Functional validation

Note that validation of algorithms and of software code are also known together as verification (Law and Kelton, 2000).

Conceptual validation concerns the question whether the model accurately represents the system under study. Was the simplification of the underlying biological process in model steps realistic; were the model assumptions credible? Usually, conceptual validation is largely qualitative and is best tested against the opinion of experts with different scientific backgrounds. (Different models

[2] Based on FAO/WHO Guidelines for Hazard Characterization of Pathogens in Water and Food, in preparation

with different conceptual bases can be tested against each other within a Bayesian framework, using Bayes factors, or some information criterion). Experimental or observational data in support of the principles and assumptions should be presented and discussed. The modelling concepts described in this document are a useful starting point, but by no means the only possible way of developing an exposure model. Many choices will inevitably be specific for a particular study, and need to be stated clearly and evaluated by independent experts.

Validation of algorithms concerns the translation of model concepts in mathematical formulas. Do the model equations represent the conceptual model, under which conditions are simplifying assumptions justified, what is the effect of the choice of (numerical) methods for model solving on the results, do results from different methods to solve the model agree? A particular point of attention for exposure models is how variability and uncertainty were implemented in the model structure. Furthermore, there are many different possibilities to mathematically describe certain steps in the exposure model. For example, different primary and secondary growth models are available, and the effect of the analyst's choice for a particular model should be evaluated.

Validation of software code concerns the implementation of mathematical formulas in computer language. Good programming practice (e.g. modular and fully documented) is an essential prerequisite. Specific points of attention are the possible effects of machine precision and software specific aspects on the model output. Internal error reports of the software are important sources of information, as well as evaluation of intermediate output. If possible, it is advisable to check the results of a new software implementation against previously published results.

Functional validation concerns checking of the model against independently obtained observations. Ideally, it is evaluated by obtaining pertinent real world data, and to perform a statistical comparison of simulated outcomes and observations. It may be very difficult, or even impossible to obtain such data. Careful evaluation of possible sources of bias in data to be used for valuation is of critical importance. It may be possible to compare results from risk assessment studies with independently obtained data on the occurrence of pathogens in the food chain. Such data can not validate the exposure model *per se* but may produce valuable insights.

Another important aspect of a model is its credibility, i.e. whether the risk manager and other key individuals accept the model as "correct" (Law and Kelton, 2000). Credibility is not synonymous to validity. Credibility can be improved by:

- The manager's understanding and agreement with the model's assumptions;
- The degree to which the model (outcomes) address the questions raised;
- Demonstrating that the model has been validated;
- The manager's ownership and involvement in the model development;
- Reputation of the model developers.

8.2 Review

The process used to develop the results can improve credibility of risk assessment results. Peer and public review of results is an essential part of the process. Interdisciplinary interaction is essential to the process of risk assessment, and should be extended to the review process. Experts in the biological processes involved should review the basic concepts and underlying assumptions used in an exposure model. Furthermore, statistical experts should review the data analysis and model construction. Critical evaluation of an exposure assessment is a demanding task that requires highly

specialised experts. Therefore, adequate resources for the peer review process should be made available as an integral part of the project plan. The results of the peer review process should be accessible to all interested parties, including a statement on how comments were incorporated in the final version of the document and if relevant reasons why specific comments were not accepted. The public review process serves two main purposes. First, it allows all stakeholders in a risk assessment to critically review the assumptions made, and their effect on the risk assessment results. Second, it allows for evaluation of the completeness of the information and datasets used for the exposure model.

8.3 Discussion and conclusions

The process used to develop the results can improve credibility of risk assessment results. Peer and public review of results is an essential part of the process. Interdisciplinary interaction is essential to the process of risk assessment, and should be extended to the review process. Experts in the biological processes involved should review the basic concepts and underlying assumptions used in an exposure model. Furthermore, statistical experts should review the data analysis and model construction. Critical evaluation of an exposure assessment is a demanding task that requires highly specialized experts. Therefore, adequate resources for the peer review process should be made available as an integral part of the project plan. The results of the peer review process should be accessible to all interested parties, including a statement on how comments were incorporated in the final version of the document and if relevant reasons why specific comments were not accepted.

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9. Conclusions

1. Quantitative risk assessment, in particular when using stochastic models, is a specialised task that requires skills in mathematics and statistics in addition to microbiological and technological knowledge. As a consequence, risk assessments are usually conducted in large, multidisciplinary projects. Building a comprehensive model may be resource intensive. The output of risk models is relatively complex, and in order to guide the risk assessment and interpret the results, risk managers need to understand the basic principles of modelling and concepts like uncertainty and variability.

2. The pivotal step in the whole risk analysis process (see Fig 2.1) is the risk evaluation step, where one identifies hazards, develops risk profiles, sets priorities and allocates resources; commissions risk assessments (including exposure assessments) and evaluate their results.

3. Exposure assessment provides an estimate of how likely it is that an individual or a population will be exposed to a microbial hazard and what numbers of organisms are likely to be ingested.

4. One should look upon the exposure assessment as an iterative and continuous process.

5. Food pathways are very complex and any model is by necessity simplification of the real world. The exposure assessment should be as simple as possible while still including the important sources of and steps leading to, the risks of concern.

6. A framework, the modular process risk model, where the process steps in the exposure assessment can be identified as one of six basic processes; growth, inactivation, partitioning, mixing, removal and cross-contamination, is suggested for the processing stages food chain.

7. The modular process risk model appears to be appropriate in the processing stage. It would be desirable to explore the possibilities for using the MPRM approach in the primary production and consumption stages.

8. The black-box approach could be useful when dealing with processes where the outcome is not critical for the results of the exposure assessment, where one is dealing with emerging issues not completely understood and where interpretation is in the observed intervals.

9. Large progress is required for predictive microbiology to be adapted to the needs of quantitative risk assessment. Limitations include that the temperature variations over time are not taken into account, since primary models not yet fully validated under non-isothermal conditions. Variability and uncertainty of model parameters are not separated. Moreover, secondary models do not enable a realistic prediction of lag times, especially after stressing conditions (such as those encountered by the bacterial population during the processing steps).

10. Preference should be given to biologically plausible models or models with biologically interpretable parameters.

11. The explicit separation of variability and uncertainty in exposure assessments should be a goal of risk assessors, and such a separation would allow decision-makers to understand how model outputs might improve if uncertainty is reduced. It also provides the risk manager more insight than working with default or worst case assumptions.

12. There is often no good match between the data available and the data needed for the exposure assessment.

13. The evolution of the methodology of quantitative risk assessment is desirable. Therefore a quick harmonisation should be avoided.

14. Despite available documents there is confusion on definitions and concepts and that should be harmonised as soon as possible at all levels e.g. OIE and Codex.

15. Peer and public review of results is an essential part of the exposure assessment process. Interdisciplinary interaction is essential to the process of risk assessment, and should be extended to the review process.

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10. Recommendations

1. Risk managers should clearly define the scope and purpose of the exposure assessment before it is commissioned, that is during the risk evaluation step.

2. Data collection strategies should be changed with a view to produce the information required for exposure assessments. Risk assessors should communicate their data needs to risk managers and risk managers should prioritize current surveillance programs to meet that need.

3. Data gaps and priority of requirements should be clearly communicated to the risk managers.

4. The strategy for dealing with data gaps should be clarified during the risk evaluation step.

5. In the meantime risk assessors will have to do their best to work with the available data and communicate the uncertainties and limitations associated with exposure assessments based upon these data.

6. One should at least for large exposure assessments always include an expert opinion step to obtain useful scenarios and reasonable estimates for likely exposures.

7. In data collection the use of meta-analyses techniques should be considered.

8. The resources when doing exposure assessments should be directed towards the most critical stages in the assessment in the relation to the risk management questions.

9. Adequate resources for the peer review process should be made available as an integral part of the exposure assessment.

10. The results of the peer review process should be accessible to all interested parties, including a statement on how comments were incorporated in the final version of the document and if relevant reasons why specific comments were not accepted.

11. The limitations in the predictive microbiology models should be addressed.

12. Traceability in the food system needs to be developed to make trustworth exposure assessments.

13. Risk assessment of microorganisms in industrial processes (with emphasis on data requirements).

14. The possibilities of using the modular processes approach for exposure assessment in primary production and consumption should be explored.

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