Food and Agriculture Organization of the United Nations

World Health Organization

AD HOC EXPERT CONSULTATIONS ON RISK ASSESSMENT OF MICROBIOLOGICAL HAZARDS IN FOODS

Report of the

Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods

FAO Headquarters, Rome, Italy

17 - 21 July 2000

Acknowledgements

The Food and Agriculture Organization of the United Nations and the World Health Organization would like to express their appreciation to the expert drafting groups (see Annex 3) for the time and effort which they dedicated to the preparation of thorough and extensive technical documents on exposure assessment and hazard characterization. The deliberations of this expert consultation were based on these documents.

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1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) convened an Expert Consultation on Risk Assessment of Microbiological Hazards in Foods in FAO Headquarters, Rome, Italy from 17 - 21 July 2000. The list of participants is presented in Annex 1.

Mr Hartwig de Haen, Assistant Director-General, Economic and Social Department in FAO opened the Consultation on behalf of the two sponsoring organizations. In welcoming the participants Mr de Haen stated that although the safety of food has always been an important issue, it is currently one that is high on the political agenda of many countries. This is due to greater consumer awareness of this issue and emerging risks and challenges in the area of food safety, one of which are microbiological hazards in foods. A number of factors have contributed to these new challenges including emerging and re-emerging pathogens, changes in methods of food production at the farm and processing level and changing consumer demands and consumption patterns.

The expansion of international trade in food has also increased the risk of infectious agents being disseminated from the original point of production to locations thousands of miles away; therefore, there was a need to address this issue at the international level. To this end Mr de Haen recalled that the Codex Alimentarius Commission (CAC), at its 23rd Session, adopted the Principles and Guidelines for the Conduct of Microbiological Risk Assessment (CAC/GL-30 (1999)). In continuing its work on microbiological risk analysis, the Codex Committee on Food Hygiene (CCFH), at its last (32nd) session, requested expert risk assessment advice on a number of pathogen-commodity combinations.

In concluding, Mr de Haen reminded the experts that they were participating in this expert consultation in their personal capacity as authoritative experts on this subject and not as representatives of their respective governments, organizations or institutions.

The consultation elected Prof. Jean-Louis Jouve (France) as Chairperson and Dr David Jordan (Australia) as Vice-Chairperson. Dr Emilio Esteban (USA) was elected as Rapporteur. The consultation also appointed a chairperson and rapporteur for each of the working groups. Dr Paw Dalgaard (Denmark) and Dr Inocencio Higuera (Mexico) were nominated as Chairperson and Rapporteur, respectively, for the working group on *Listeria monocytogenes* in ready-to-eat foods. Dr David Jordan and Dr Julia Kiehlbauch (USA) were nominated as Chairperson and Rapporteur, respectively, for the working group on *Salmonella* spp. in eggs and broilers.

2. Background

Risk assessment of microbiological hazards in foods has been identified as a priority area of work for the CAC. In 1999, FAO and WHO convened an expert consultation in Geneva, addressing for the first time the issue of risk assessment of microbiological hazards in foods. The consultation developed an international strategy

and identified mechanisms required to support risk assessment of microbiological hazards in foods. As a follow-up to that consultation and in response to the request of the CCFH, FAO and WHO have jointly embarked on a programme of activities with the objective of providing expert advice on risk assessment of microbiological hazards in foods to their Member Countries and to the CAC (see Annex 2).

Dr. Jorgen Schlundt, Coordinator, WHO Food Safety Programme, outlined the background for the development of the food safety risk analysis framework and the evolution of international microbiological risk assessments through FAO, WHO and Codex initiatives over the last decade. He emphasised that the present expert consultation represents the initiation of the international work on microbiological risk assessment of specific pathogen/commodity combinations as suggested by the 32nd session of the CCFH and is important for FAO and WHO Member Countries and the CAC. Furthermore Dr. Schlundt stressed that this initiative may be considered as a cornerstone for future food safety improvements, both at the national and the international level.

Ms. Maria de Lourdes Costarrica, Senior Officer, Food Quality Liaison Group, FAO informed the expert consultation that the purpose of this meeting was to provide expert advice and guidance to FAO and WHO Member Countries based on an evaluation of the available information on risk assessment of three pathogen-commodity combinations; *Salmonella* spp. in [*]broilers; *Salmonella* Enteritidis in eggs and *Listeria monocytogenes* in ready-to-eat foods. Temporary expert drafting groups were jointly established by FAO and WHO to examine this information and prepared technical papers that were presented to the consultation for review and discussion. In addition to these, draft guidelines for hazard characterization of pathogens in food and water prepared during a WHO/FAO/RIVM Workshop on this subject held in Bilthoven, the Netherlands on 13 – 17 June 2000 were also presented for consideration by the expert consultation (Annex 3).

3. Objectives of the Consultation

The consultation examined the technical documents on hazard characterization and exposure assessment of *Salmonella* spp. in broilers and eggs and *L. monocytogenes* in ready-to-eat foods, and the draft guidelines on hazard characterization with the following objectives:

1. To provide scientific advice to FAO and WHO Member Countries and Codex on the risk assessment of *Salmonella* spp. in broilers and eggs and *L. monocytogenes* in ready-to-eat foods.

2. To provide guidance to FAO and WHO Member Countries and Codex in the form of practical guidelines and methodology for hazard characterization of microbial pathogens.

[*] Broilers: young chickens that are reared specifically for the poultry processing industry

3. To identify the knowledge gaps and information requirements needed to complete the above-mentioned risk assessments.

4. Summary of the General Discussions

The consultation noted that microbiological risk assessments could have a wide range of applications in food safety. Ideally, a risk assessment should encompass all components of the food system from production to consumption, so that risk factors as well as different strategies to reduce risks can be thoroughly described. A microbiological risk assessment can be used for a number of purposes such as, to develop broad food safety policies, develop sanitary measures that achieve specific food safety goals, and elaborate standards for food.

FAO and WHO are, over the next two years, undertaking a summary and interpretation of risk assessments for three pathogen-commodity combinations identified as priorities by the CCFH (ALINORM 01/13). The consultation recognized that in the absence of specific risk management guidance from the CCFH, the approach taken by FAO and WHO and the expert drafting groups in developing hazard characterizations and exposure assessments for *Salmonella* spp. in broilers and eggs and *L. monocytogenes* in ready-to-eat foods was comprehensive and all embracing rather than being tailored to address specified risk management questions. Although an approach tailored to a specific question is preferred, the comprehensive approach taken does advance international understanding of two important components of risk assessment in a broad sense, and provides a strong platform for future provision of risk assessment advice as requested by FAO/WHO Member Countries, the CCFH, and other stakeholders.

The primary purpose of this report is to present updated executive summaries of the above-mentioned hazard characterizations and exposure assessments documents as prepared by the expert drafting groups, summarize the technical discussions arising from the presentation of these topics to the expert consultation, and make recommendations for further technical development. During the consultation the executive summaries presented with the technical documents were amended as appropriate for inclusion in the report. Where time allowed some amendments were made to the technical papers. The record of the deliberations of the relevant working groups presented here includes issues to be brought to the attention of FAO and WHO. Recommendations to facilitate this process, in both a specific and a general sense, are made to FAO and WHO. The report provides a transparent review of scientific opinion on the "state of the art" of microbiological risk assessment and identifies gaps in the data that need to be filled if sound quantitative risk assessments of the pathogen-commodity combinations specified by the CCFH are to be achieved. The report also illustrates the difference in modelling and resourcing demands for particular risk assessments according to the purpose and scope defined by risk managers.

The technical documents prepared by the expert drafting groups will be further revised to take into account public comment and ongoing input from the Joint Expert Consultations on Risk Assessment of Microbiological Hazards in Foods. The

intention is to use this work to prepare a full risk assessment document pending further advice regarding scope and presentation from the CCFH.

5. Hazard characterization and exposure assessment of *Salmonella* spp. in broilers and eggs.

The technical documents on *Salmonella* hazard identification, hazard characterization and exposure assessment presented to the consultation were discussed in detail by working groups. The full documents are available on request from FAO or WHO and can also be found at the following Internet addresses: http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/pagerisk/riskpage.htm and http://www.who.int/fsf/mbriskassess/index.htm.

The executive summaries of these documents were updated during the consultation to take into account some of the questions and comments on the papers resulting from these discussions and are presented below. These are followed by a summary of the discussions of additional points that were not directly incorporated into the executive summaries of the discussion papers.

5.1. HAZARD CHARACTERIZATION OF SALMONELLA IN BROILERS AND EGGS

5.1.A Executive summary

Introduction

This document focuses on evaluating the nature of the adverse health effects associated with foodborne non-typhoid and non-paratyphoid *Salmonella* spp. and how to quantitatively assess the relationship between the magnitude of the foodborne exposure and the likelihood of adverse health effects occurring.

Objectives

The objective and scope of the *Salmonella* Hazard Characterization document is to provide:

- A review of the characteristics of the host, organism and food matrix.
- A summary and review of available data and information on adverse health effects.
- A summary and evaluation of existing dose-response models with respect to assumptions, sources of uncertainty, strengths and limitations.
- A description of the use of available outbreak data to evaluate published dose-response models.
- An evaluation of the outbreak data for evidence of a difference between susceptible and normal populations and between *Salmonella* Enteritidis and other strains.

Approach

Information was compiled from published literature and from unpublished data submitted to FAO/WHO by public health agencies and other interested parties. The first section of the document provides a description of the public health outcomes, pathogen characteristics, host characteristics, and food-related factors that may affect the survival of *Salmonella* in the human gastrointestinal tract.

The second section of the hazard characterization document presents a review of the background and rationale for different models that have been reported and used to estimate the dose-response relationship of *Salmonella*. These models mathematically describe the relationship between the numbers of organisms that might be present in a food and consumed (dose), and the human health outcome (response). There are three different models that have been published or reported: the USDA/FSIS-FDA *Salmonella* Enteritidis model, the Health Canada *Salmonella* Enteritidis model, and a beta-Poisson model fit to human feeding trial data for various *Salmonella* species.

An extensive review of available outbreak data was also conducted and data appropriate for dose-response estimations were summarized. The dose-response curves reviewed were then compared with the outbreak data to equate the model with observed information. Where possible, the outbreak data were also used to characterize the differences that may exist between the potential for infection in susceptible and in normal segments of the population. Finally, the outbreak data were used to estimate additional dose-response models.

Overall, the document on "Hazard identification and hazard characterization of *Salmonella* in broilers and eggs" provides a summary of a vast amount of literature available on this subject.

Key findings

In most people the gastroenteritis resulting from salmonellosis lasts 4 - 7 days and patients fully recover without medical treatment. However, some people may develop more severe illness, including potentially fatal infections or long-term syndromes such as reactive arthritis and Reiter's syndrome.

Clinical manifestations of *Salmonella* infections in animals generally differ from the typical gastroenteritis and other sequelae produced in humans, therefore, extrapolations of disease in animals to disease in humans must be done with great caution.

In the case of *Salmonella*, unlike most other bacterial pathogens, there is a reasonable amount of human data. As a result, it was believed that the inclusion of additional information from animal data may contribute to increasing the uncertainty rather than improving the dose-response relationship.

Insight into the potential for some segments of the population to be more susceptible to *Salmonella* infection than others was provided by data extracted from two outbreaks. Assuming children under 5 years of age represented a more susceptible population, it was estimated that at the doses observed in these outbreaks

(approximately 2 and 4 log CFU/g), the susceptible population was 1.8 to 2.3 times more likely to get ill.

A review of currently available outbreak data did not produce any evidence to support the hypothesis that *Salmonella* Enteritidis has a higher likelihood of causing illness upon ingestion than a similar dose of another serovar.

The outbreak data indicate that the dose-response relationship for all non-typhoid and non-paratyphoid *Salmonella* spp. can be characterized using a single dose-response model.

While there is a reasonable amount of human data, complete outbreak data are sparse and important information for the calculation of dose-response assessments is often missing from outbreak reports. In particular, enumeration of organisms in the implicated food vehicle is frequently not carried out in many outbreak investigations. Valuable data for this report was provided by Japan[*], where since 1997, all large foodservice establishments have been advised to keep frozen portions of prepared foods for a minimum of 2 weeks for subsequent testing if illness is associated with the food. These data allowed significant insights to be made into the hazard characterization of *Salmonella*.

Five models are summarized below and in Figure 5.1. Three models are published or documented in official reports and two new models were generated from the collected outbreak data. They are:

i. Naïve human feeding trial data beta-Poisson model

The model suffers from the nature of the feeding trial data (i.e. the subjects used were healthy male volunteers) and may not reflect the population at large. The model tends to greatly underestimate the probability of illness as observed in the outbreak data, even if the assumption is made that infection, as measured in the dose-response curve will equate to illness.

ii. USDA/FSIS-FDA *Salmonella* Enteritidis beta-Poisson model

The model uses human feeding trial data for *Shigella dysenteriae* as a surrogate pathogen with illness as the measured endpoint in the data. The appropriateness of using *Shigella* as a surrogate for *Salmonella* is questionable given the nature of the organisms in relation to infectivity and disease. Compared to the outbreak data, and on a purely empirical basis, this curve does tend to capture the upper range of these data.

iii. Health Canada *Salmonella* Enteritidis beta-Poisson model

To date this model has not been fully documented and lacks transparency. The model uses data from many different bacterial pathogen-feeding trials and combines this information with key *Salmonella* outbreak data using bayesian

[*] In accordance with Japanese notification released on March 1997, large scale catering facilities (> 750 meals per day or > 300 dishes of a single menu) have been advised to save food for future examination in the case of illness being associated with the food. Fifty gram aliquots of each raw food material and cooked dish should be saved for a minimum of 2 weeks at temperatures below –20 ºC. Although this notification is not mandatory, it is also applicable to smaller catering facilities with social responsibility such as those in schools, day-care centres, and other child-welfare and social-welfare facilities. Some local governments also have regulations relating to food saving, but the required duration and the temperature of storage vary.

techniques. Using data from many bacterial feeding trials and the current lack of full documentation regarding their role is a point of caution. Empirically, the curve describes the outbreak data at the low dose well but it tends towards describing the lower range of response at higher doses.

iv. Outbreak data exponential model

The exponential model fit to the outbreak data does not produce a statistically significant fit. The curve does provide an adequate description of the data at the mid- and high-dose range; however, it underestimates the low-dose observed data.

v. Outbreak data beta-Poisson model

The beta-Poisson model similar to the exponential model fit to the outbreak data does not produce a statistically significant fit. The curve does produce an adequate characterization of the observed data in the low to mid-dose range. The low dose range of the dose response relationship is an especially important area.

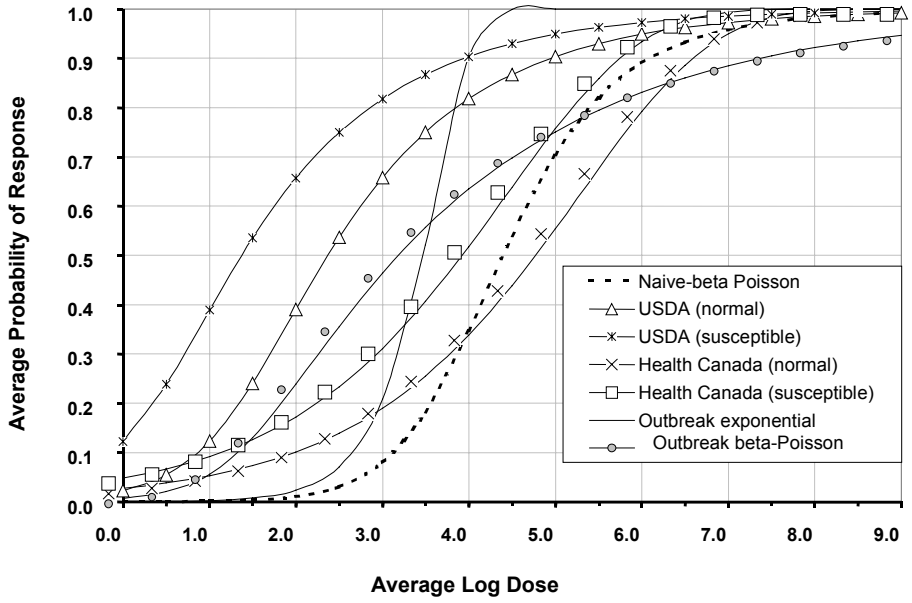

Figure 5.1: Comparison of *Salmonella* dose-response models.
NOTE: The points on the curves do not represent data points and are used only for legend purposes.

Gaps in the data

- Outbreak and epidemiological data, specifically indicating: concentration in the implicated food, amount of food consumed, accurate numbers on ill and exposed populations, accurate characterization of the population including age profiles, medical status, sex and other potential susceptibility factors.
- Quantitative data measuring the impact of the food matrix effects on the probability of infection.
- Quantitative information to facilitate estimating the probability of developing sequelae following illness.

- Characterization and quantification of the relationship between susceptibility factors and increased likelihood of infection.

Conclusions

The derivation of any one of the models is based on many assumptions, such as the use of *S. dysenteriae* or other surrogates for *Salmonella*, combining results of feeding studies for different pathogens, the relevance of infection versus illness as endpoints, and the study design and health status of the test subjects in the human feeding trials. The outbreak data revealed several uncertainties and several assumptions had to be made to derive some of the outbreak estimates subsequently used to fit new dose-response curves.

At present, a single model representation for the relationship between dose and response can not be highlighted as vastly superior to any other model. Compared to the reported outbreak data, the naïve beta-Poisson model is the least desirable since it vastly underestimates the probability of illness and tends towards the lower bound even when the assumption is made that all infections lead to illness. The remaining models were relatively reasonable approximations, with different degrees of under- or over-prediction of illness based on the outbreak data described in this report. The models fit to the outbreak data appear to offer reasonable potential given that they qualitatively, though not statistically significantly, describe observations in a real world environment.

Recommendations

- Consideration should be given to the inclusion of *S. typhi* and *S. paratyphi* in future hazard characterizations. A dose-response relationship for all *Salmonella* spp. could prove to be of great utility, and the added information from *S. typhi* could also serve to expand the current information
- This document did not consider a quantitative evaluation of secondary transmission (person-to-person) or chronic outcomes. In addition, the impact of the food matrix was not incorporated into the assessment. These may be considerations for future document development.
- Additional data will help to refine the current information and ideally support the development of better risk assessments to help make more accurate predictions regarding the safety of foods contaminated with *Salmonella* and other pathogens of public health concern.
- The importance of accurate and complete epidemiological data collection during outbreak investigations should also be communicated and encouraged

5.1.B Summary of discussions related to hazard identification and hazard characterization of *Salmonella*

General Comments

The expert consultation welcomed the technical report prepared by the expert drafting group as a significant advancement towards the understanding of the hazard

characterization of *Salmonella*. It was recommended that the title of the document should be changed to "Hazard identification and hazard characterization of non-typhoid and non-paratyphoid *Salmonella*" to better reflect the scope of the work.

Dose response curves

The consultation agreed that the inherent variability of the *Salmonella* dose-response data summarized in the report necessitated the fitting of several dose-response curves to describe the outbreak observations. Evidence does not support the selection of a single curve for summarizing the *Salmonella* dose-response data at this time. Given the limited number of data sets and reliability/variability of the data, it was decided to retain all candidate dose-response curves and provide commentary on the degree of fit, practical suitability, and pros and cons of each curve.

The appropriateness of using *Shigella* as a surrogate for *Salmonella* is questionable given the nature of the organisms in relation to infectivity and disease. However, compared to the outbreak data, and on a purely empirical basis, this curve does tend to capture the upper range of the outbreak data.

Specific comments related to dose response curves based on outbreak data

The dose-response data from outbreaks were highly relevant to foodborne illness but models produced using available data had a poor statistical fit. There were limitations to the outbreak data as the data were collected over several decades and the investigative methodology may have changed without including corrections to account for methodological changes. It was noted that the majority of the data were from North America, Europe and Japan. General applicability of outbreak data dose-response assessments would be improved by including data from other countries.

Much discussion focused on the advantages/disadvantages of deleting particular observations because they did not fit the general trend of the outbreak data (see Figure 5.2 - outliers). The requirement to be transparent dictates that when data are excluded the authors/modellers should be explicit regarding the reasons for this.

It was suggested that a linear regression of attack rate on the $\log_{10}$ number of *Salmonella* consumed might provide an alternative description of the dose response relationship. Such a model would have the advantage of simplicity but was not preferred to beta-Poisson type models that reflect an underlying biological basis of infection. It was noted that the linear regression approach could disguise the truly non-linear relationship between concentration and attack rate.

The present models do not account for food matrix effects nor fully account for host or pathogen variations. One example of a host effect that is not captured in the model is treatment with antibiotics that may make an individual more susceptible to infection because of changes in the composition of the gut flora. It was noted that good calibration studies would help identify how to consistently adjust specific attack rates for individual outbreaks.

It was noted that *Salmonella typhi* and *Salmonella paratyphi* outbreaks were not included in the data used to generate the present model. Experts agreed a hazard characterization that includes *S. typhi* and *S. paratyphi* data should be considered in the future.

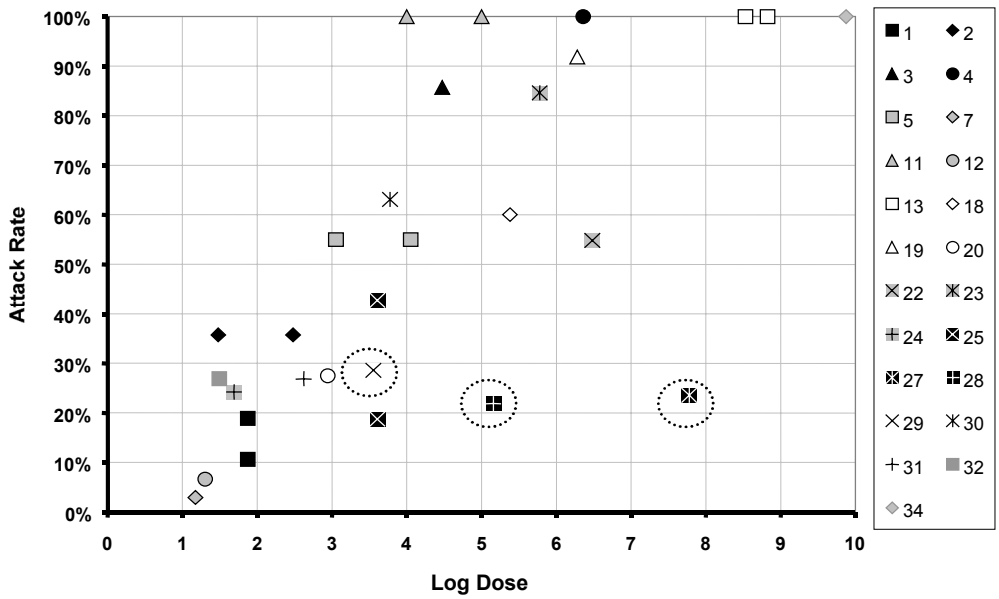

Figure 5.2. Outliers. The three data points circled are deleted for modelling outbreak data because they did not fit the trend of the data. For further discussion of the rationale behind this, see the full document referenced below.

Note: Numbers 1 - 34 in the legend refer to outbreak data. These outbreaks are described in detail in the text of the document on Hazard identification and hazard characterization of *Salmonella* in broilers and eggs available on the Internet:

http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/pagerisk/riskpage.htm and
http://www.who.int/fsf/mbriskassess/index.htm

Gaps in the data

- Specific information regarding incidence of disease in countries other than North America, Europe, and Japan. This may require the use of regional centres for collection of this information.

- Studies on the microbial ecology to determine reservoir and quantity of organism would support hazard identification.

Recommendations

- WHO should review and update specific guidelines to ensure more thorough and consistent investigation of outbreak data so that these data are available for hazard characterization. Specific guidelines are needed to address the problems of measurement error in data (including both the accuracy of bacterial enumeration and determination of the number of individuals exposed), variations in case definitions of illness for different outbreak investigations, and better identity of suspected/incriminated foods. This should also include guidelines for selecting data sets used to determine dose-response relationships including recommendations regarding sampling, laboratory methods, epidemiological methods and analysis.

- FAO/WHO should strongly encourage Member Countries to publish existing and new epidemiological data and to conduct epidemiological investigations

of outbreaks with data that include enumeration of pathogens and denominators for attack rates.

- FAO/WHO are encouraged to facilitate the development of protocols used to measure attack rates and collection of surveillance data.

- The consultation recommends to FAO/WHO that prevalence and outbreak data be compiled in a central repository.

- FAO/WHO should facilitate the development of methods suitable for enumeration of salmonellae in foods including identification/development of new enumeration techniques.

- Current models should be expanded to include food matrix and host effects.

5.2. EXPOSURE ASSESSMENT OF SALMONELLA ENTERITIDIS IN EGGS

5.2.A Executive summary

Introduction

A critical review of the existing exposure assessment models can contribute to the advancement of microbiological risk assessment. Discussions between the FAO/WHO secretariat and the expert drafting group determined the need for a comparison of existing exposure assessments to characterize the state of the art in the practice of risk assessment. Such a comparison would identify similarities and differences between existing models. This approach should be beneficial to future exposure assessments of this pathogen-commodity combination.

The review intends to identify those methods that were most successful in previous exposure assessments, and also recognize the weaknesses of those assessments as a result of inadequate data or methodology. Although no specific risk management direction was provided for this report, the findings should be useful for future risk management.

Objectives

The purpose of this report is to compare existing techniques and practices used to construct an exposure assessment for *Salmonella* Enteritidis in eggs and to provide a framework for future exposure assessments of this pathogen-commodity combination.

The scope of this analysis is limited to the probability of human exposure associated with eggs that are internally contaminated with *Salmonella* Enteritidis. The analysis and conclusions are similarly focussed to only apply to currently understood mechanisms and variables as used in previous exposure assessments. Therefore, caution should be exercised in interpreting this report in relation to data that has become available since these models were completed.

Approach

Five previously prepared exposure assessments of *Salmonella* Enteritidis in eggs were reviewed. Of these, three exposure assessments were selected for in-depth comparisons.

These were:

- **USDA/FSIS-FDA.** *Salmonella* Enteritidis risk assessment: Shell eggs and egg products. Final Report. June 12, 1998.

- **Health Canada**. *Salmonella* Enteritidis risk assessment model. Unpublished.

- **Whiting R.C. and Buchanan R.L. (Whiting)**. Development of a quantitative risk assessment model for *Salmonella* Enteritidis in pasteurized liquid eggs. International Journal of Food Microbiology 36:111-125, 1997.

Four stages of a "farm-to-table" exposure assessment were defined: production, distribution and storage, egg products processing, and preparation and consumption. The production stage considers the laying of *Salmonella* Enteritidis contaminated eggs. The distribution and storage stage considers the time between lay and preparation of egg-containing meals. The egg products processing stage considers commercially broken eggs that are usually pasteurized. Preparation and consumption stage considers the effects of different meal preparation practices and cooking.

The USDA/FSIS-FDA exposure assessment included all above four stages of an exposure assessment. The Health Canada model included production, distribution and storage, and preparation/consumption, but did not cover egg products processing. The Whiting model focused on egg products processing, but it also included elements of production and distribution/storage stages.

Generally, data considered in this analysis applies to either occurrence or concentration of *Salmonella* Enteritidis. Specific data used in previous exposure assessments are presented and analysed for each of the model stages. To provide a more complete description of available data, a summary of published and non-published research on *Salmonella* Enteritidis occurrence and concentration was undertaken. Although some of these data are not used in the three previously prepared exposure assessments, their inclusion in the report provides currently available data that could assist future exposure assessments.

Key findings

Accurate estimates of prevalence inputs require that surveillance data be adjusted to account for likelihood of detection and other biases. The USDA/FSIS-FDA model includes such adjustments, but the other two models did not.

In the distribution and storage stage, there is a need to separately model growth for each distinct pathway to account for different time and temperature distributions. Growth of S*almonella* Enteritidis inside eggs was found to be sensitive

to assumed temperature distributions at retail and consumer storage in two exposure assessments. When time and temperature inputs are similarly defined, the USDA/FSIS-FDA and Health Canada models give similar predictions (see Figure 5.3).

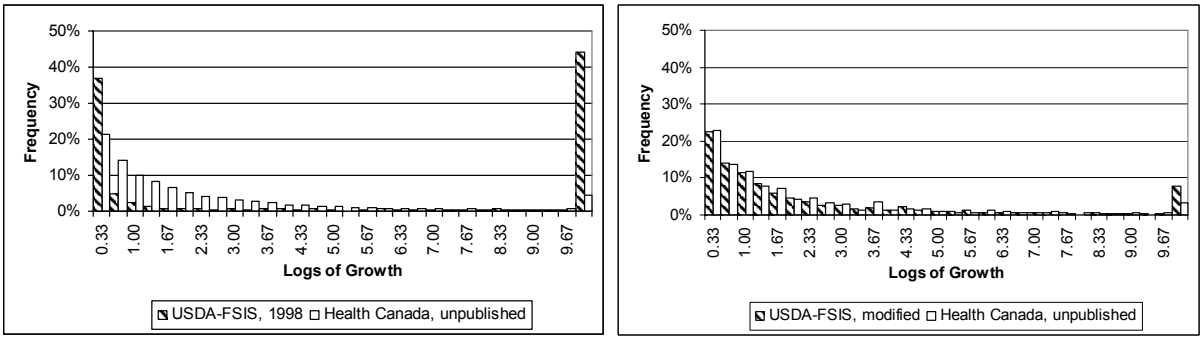

Figure 5.3. Comparison between USDA/FSIS-FDA and Health Canada models. On the left are predicted distributions of logs of growth for those contaminated eggs in which growth occurs. On the right are these predictions when the USDA/FSIS-FDA model temperature inputs are modified to be similar to the Health Canada model inputs.

The USDA/FSIS-FDA and Whiting models of the egg products processing stage predicted wide variability in pasteurization effectiveness. This finding substantially influences the predicted number of *Salmonella* Enteritidis remaining in egg products after pasteurizing.

The USDA/FSIS-FDA model predicts an increased probability of exposure associated with pooling of eggs in the preparation stage, while the Health Canada model shows a decrease in probability of exposure associated with egg pooling. This difference occurs because the Health Canada model does not include post-pooling growth and restricts pooling scenarios to those only involving scrambled egg meals. If more diverse pooling scenarios were considered in the Health Canada model, then pooling might more significantly contribute to probability of illness in that model.

Gaps in the data

Data relating to the ecology of *Salmonella* Enteritidis in eggs are needed. This need is seemingly universal in its application to previous and future exposure assessments.

- It was recognized that estimating the number of *Salmonella* Enteritidis contaminated eggs at the production stage was based on data from, at most, 63 eggs. More epidemiological and enumeration data would improve modelling of egg contamination.

- To adequately assess preharvest interventions, more data are needed on the prevalence of *Salmonella* Enteritidis in breeder and pullet flocks, as well as in feedstuffs. In particular, associations between the occurrence of *Salmonella* Enteritidis in these preharvest steps and its occurrence in commercial layers should be quantified.

- Better data on time and temperature, specifically in relation to egg storage would serve to build confidence in modelling. The importance of time and temperature distributions in predicting growth of *Salmonella* Enteritidis in eggs – combined with the lack of reliable data to describe these distributions – highlights the need for these data.

- The high degree of uncertainty/variability in cooking effectiveness inputs noted in the comparison of the models also highlights the need for more research on these inputs.

- To predict reliably the effectiveness of regulatory standards concerning egg products, there is a need for additional data concerning the concentration of *Salmonella* Enteritidis in raw liquid egg before pasteurization.

The exposure assessments considered in this report primarily relied on relevant North American data. Additional data will need to be collected to conduct exposure assessments in countries where egg contamination with *Salmonella* Enteritidis is different to that in North America. For example, countries will probably need to assess the prevalence of *Salmonella* Enteritidis in their egg industry. The marketing fractions, times and temperatures of storage, and preparation and cooking practices will probably differ in other countries. Therefore, these exposure assessment inputs will need to be estimated from country- or purpose-specific data.

Conclusions

This report identifies similarities and differences between previously prepared exposure assessments of *Salmonella* Enteritidis in eggs. Potential pitfalls, important data analysis, and critical data needs are reported for each stage of a "farm-to-table" exposure assessment. This report does not intend to provide detailed guidelines on how to conduct an exposure assessment of this pathogen-commodity combination. Additional work is required to develop such guidelines. In addition to this report's findings, those wishing to complete such an analysis should refer to the original papers cited in the report, as well as risk analysis texts.

Many similarities were found in the approaches used by the three exposure assessments analysed in this report. For example, the distributions for initial number of *Salmonella* Enteritidis per egg were derived in a similar manner. The growth equations were similar, as were the pasteurization equations. Often, the same distribution types were used to model the same inputs, although different parameters might be specified. The modelling approaches – for example the pathways considered, and the factors modelled – were very similar.

It was concluded that *Salmonella* Enteritidis exposure assessments should model growth and preparation/consumption as one continuous pathway. In this manner, growth and decline of *Salmonella* Enteritidis is explicitly modelled as dependent on the pathway considered.

Predictive microbiology should be common to any exposure assessment of *Salmonella* Enteritidis in eggs. Because environmental conditions differ on an international level, time and temperature distributions may be different between

analyses. Yet, it was concluded that the predictive microbiology equations used in future exposure assessments could be similar.

Careful attention should be placed on areas in preparation and consumption where the product changes form or the units change. Pooling eggs into a container creates a product distinctly different from shell eggs. This product is able to support immediate bacterial growth and its storage should be modelled as a unique event.

Given the lack of published evidence on relevant egg consumption and preparation practices among populations of end-users, the preparation and consumption component of an exposure assessment is the most difficult to accurately model. Even with perfect information, this component is very complicated. Multiple pathways reflecting multiple end-users, products, practices, and cooking effectiveness levels ensure that the preparation and consumption component has many difficulties. Nevertheless, the strides taken in previous models can serve as reasonable starting points for subsequent analyses.

Limitations common to the models compared in this report include lack of consideration for possible re-contamination of egg products following pasteurization and/or cooking, and no consideration of cross-contamination of other foods from *Salmonella* Enteritidis contaminated eggs. Furthermore, the results and conclusions of these models are dependent on conventional assumptions regarding mechanisms of egg contamination. These mechanisms suggest that *Salmonella* Enteritidis contamination in eggs is initially restricted to albumen and that such contamination enters eggs during their formation inside hen's reproductive tissues. Also, the growth kinetics estimated for these models are not necessarily representative of all *Salmonella* Enteritidis strains or other *Salmonella* serotypes.

While these models are similar to one another, and provide common stages of an exposure assessment, they may require substantial reprogramming to be useful to some countries or regions where the situation is markedly different from that in North America. Such reprogramming may be limited to changing some input distributions, but may also require eliminating or adding some variables or parameters to the models.

Recommendations

- The scope of an exposure assessment should be clearly defined and its objectives clearly stated.

- The existing exposure assessment models – in combination with dose-response models developed for *Salmonella* species - can be used to evaluate some mitigation strategies, but the applicability of their findings would most likely be limited to the countries for which the model inputs were derived.

- Because the predictive microbiology of *Salmonella* Enteritidis in eggs is considered common to any exposure assessment of this pathogen-commodity combination, its progress and further development are encouraged. A central repository of data on the mathematical behaviour of

Salmonella Enteritidis in eggs should be established. FAO and WHO should consider supporting this initiative.

- In parallel with the current work to develop guidelines for hazard characterization, FAO/WHO should facilitate the development of guidelines for exposure assessments, including approaches to be used when data are limited or only semi-quantitative in nature.

5.2.B Summary discussion related to exposure assessment of *Salmonella* Enteritidis in eggs

General Discussion

The consultation welcomed the technical report as an important contribution on exposure assessment of *Salmonella* Enteritidis in eggs. It agreed that inclusion of epidemiological concepts in determining flock prevalence, within flock prevalence, and apparent prevalence was a significant feature of this study.

Current models for exposure assessment are used to predict the public health benefits of an intervention imposed at any time prior to consumption. However, data may not be available for assessing all candidate interventions. If the concern is at consumption, data prior to this are not required. In contrast, the assessment of interventions applied in the pre-harvest period demand data is available for a larger segment of the production-consumption process.

Limitations of the models presented are that they do not allow a discussion of cross-contamination and recontamination although anecdotal evidence suggests that these are important. The models are not specifically designed to evaluate the importance of vertical transmission in breeder flocks. The consultation noted the need to consider this and the geographic variation of flock prevalence.

The current models also place emphasis on the potential for growth of *Salmonella* Enteritidis in eggs. The consultation noted that growth was not always necessary for human infection, as very low doses can be infectious. Although published information does not indicate a difference in heat sensitivity between *Salmonella* Enteritidis and other serotypes, recent evidence suggests that specific strains (e.g. *Salmonella* Enteritidis PT4 containing a 25 mD plasmid) may have differing growth characteristics.

Robust testing and sampling methodologies are essential for exposure assessments. For example, when considering pooled eggs, small numbers of organisms present in the eggs may reduce the probability of detection.

The models assume that *Salmonella* in naturally infected eggs are located in the albumen outside the vitelline membrane. The models also assume there is a brief period of time immediately following lay where there may be growth of *Salmonella* Enteritidis in the egg. This assumption may not be valid in all cases. Anecdotal data suggests there may be conditions where *Salmonella* grows rapidly in intact eggs.

The breakdown of the yolk membrane is a key concept of the model. However, some experts expressed a cautionary note on the simplification introduced by modelling the lag phase in cumulative fashion. Assumptions such as this may be necessary for simplicity of the model; however, they result in a less than precise depiction of the real world situation.

Gaps in the data

- Additional studies on the number of *Salmonella* Enteritidis in naturally contaminated intact shell eggs are required (information is currently available for 63 intact shell eggs). There is also a need for enumeration data of *Salmonella* Enteritidis in raw liquid egg.

- Additional data are required on the duration of storage in retail stores and in homes, and on temperatures experienced during those storage periods.

- Information is required on the characteristics of flocks in different countries. This information would include flock age profiles and the size of flocks.

- Information is required on individual preferences for storage and consumption in different countries including information regarding the actual cooking practices used. For example, time between lay and consumption differs between the US and Canada. This important variable must be determined for each individual country.

- More inoculation studies are required to assess survival time of *Salmonella* Enteritidis in eggs.

- Additional studies are required to estimate the kinetics of growth as a function of egg composition and strain infectivity as well as heat sensitivity between various *Salmonella* Enteritidis strains.

- Studies need to be conducted on sites of *Salmonella* Enteritidis infection in the reproductive tract of hens.

- Studies are required to investigate the possibility that under certain conditions *Salmonella* can grow rapidly in intact eggs.

5.3. EXPOSURE ASSESSMENT OF SALMONELLA SPP. IN BROILERS

5.3.A Executive summary

Introduction

An understanding of *Salmonella* spp. in broilers is important from both public health and international trade perspectives. As a result, there is an urgency to evaluate this pathogen-commodity combination by quantitative risk assessment methodology.

To date, no full quantitative exposure assessments have been undertaken in this respect. This work illustrates a way that such assessments can be developed.

Objectives

This report focuses on the development of a model framework, highlighting ideal data requirements and possible methodologies. In addition, it presents available data for developing such models and makes an assessment of their usefulness. It is not intended to present a full farm-to-fork model; rather, the content of the report can be used for guidance. Where appropriate, example models are presented to illustrate possible methodologies related to individual steps that could be included within a full model.

Considering the proposed methodologies and available data, areas of limited information are highlighted and recommendations for directing future study are made.

Approach

The report begins by presenting an overall model framework that describes the exposure pathway from the farm to the point of consumption (see Figure 5.4). The pathway consists of a number of related modules (production, transport and processing, retail, distribution and storage, preparation) that describe the changes in prevalence and concentration of organisms. If the framework is used to construct a model, the outputs can then be combined with consumption data to estimate exposure.

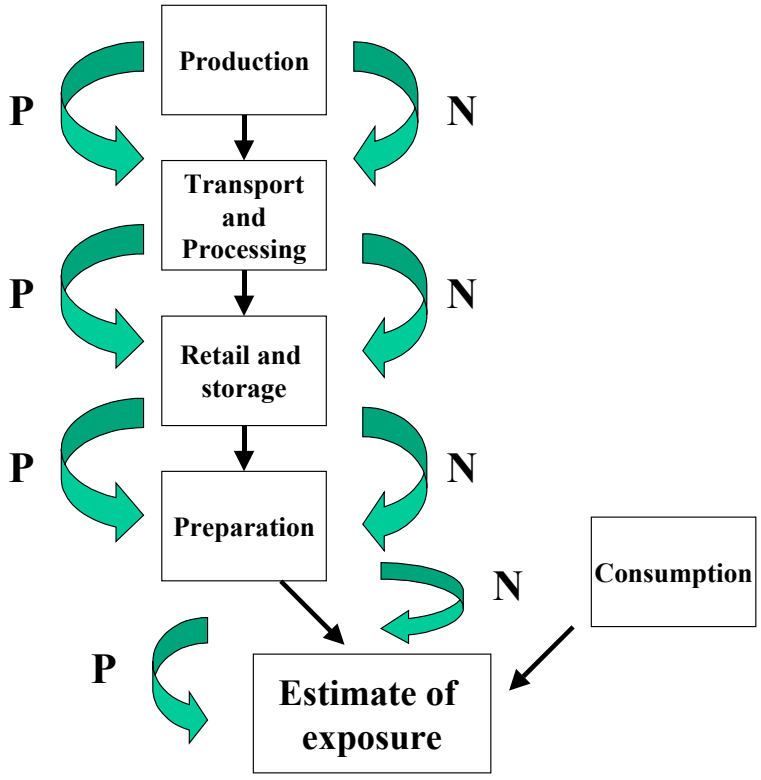

Figure 5.4: Modular pathway to describe the production to consumption pathway.
P: Changes in prevalence
N: Changes in numbers of organisms

Issues common to all steps on the pathway are discussed. In particular, data related factors are explored. These factors include possible data sources (published, regulatory and industry), problems associated with obtaining data from different sources, combining data from disparate sources and selecting the most valid data. Different modelling approaches are also summarized including the use of static and dynamic models, deterministic and stochastic alternatives and the appropriate incorporation of uncertainty and variability. With these points in mind, the individual modules are considered in detail.

The production module aims to estimate the prevalence of *Salmonella* positive broilers at the time of leaving the farm for slaughter. The number of organisms per positive bird is a required output. Ideal data requirements for this step are outlined and include source of infection, flock prevalence, within flock prevalence and full details of study methods including sampling (e.g. site, selection, timing, relationship to overall population) and microbiological methods used. The number of organisms per bird is also essential.

Processing of broilers is outlined in the second module and here the aim is to estimate prevalence and concentration at the end of processing. For this module, ideal data relates to changes in numbers and prevalence during the various steps of processing, together with details of the study as discussed previously. Such information should capture the importance of cross-contamination during this step.

Retail, distribution and storage (module 3) considers the time after processing and before preparation and consumption by the consumer. The aim is to estimate the change in the number of organisms per contaminated product. These stages can be considered as a series of time / temperature profiles to which the broiler is exposed and, therefore, growth and survival are the critical microbial processes. There are two classes of ideal data for this module. Firstly, the time / temperature data which describes the processes and secondly appropriate predictive models to describe the growth and survival processes.

Preparation is considered in module 4 to estimate the change in numbers as a result of preparation prior to consumption. Ideally, cross-contamination should be modelled in this stage and thus appropriate models and data are required for this module. In addition, when considering frozen broilers, data are needed to describe the thawing process. Finally, data relating to cooking are required for use in predictive models that describe thermal death.

In the final module consumption patterns are considered. Ideally, this requires data on consumption patterns of a population. To be useful, the population should be divided into sub-groups that could be based, for example, on age, sex or immune status, etc. Consumption data must be national - generalisation is not appropriate.

Although no full exposure assessments have been undertaken for this pathogen-commodity combination, there are models available that start later in the exposure pathway (e.g. the start of processing and retail). A full exposure assessment for *Campylobacter* in broilers has been undertaken. These assessments are reviewed to determine their usefulness for a full exposure assessment of *Salmonella* in broilers

(recognising the differences between *Campylobacter* and *Salmonella*). From this review, many of the models have features that could be utilised.

Key findings

Modelling the full exposure pathway from farm-to-fork is a complex process. The individual modules of this pathway will be complex and may have high degrees of associated uncertainties which, when combined, can generate an estimate of exposure with a wide range of uncertainty. Consequently, it is important to consider the points where modelling should begin and end. This will be defined by the risk management question.

When collating data from a large number of dissimilar studies, it is important to present this information in tabulated form, considering the ideal data requirements identified prior to collection. Such presentation enables critical evaluation of the data and helps to ensure that the most valid data are selected.

With regard to models for individual stages of the exposure assessment, there is a balance between the need for accurate prediction and the simplicity of the approach taken. This should be considered during the model selection process.

Gaps in the data

The main gaps identified in the data are as follows:

- There is limited prevalence data for many regions of the world and for areas where prevalence is reported information on the study design is often lacking.
- For all stages of the exposure pathway, there is little recent quantitative data on the numbers of organisms per bird.
- Cross-contamination data is extremely scarce and modelling of this event is in its infancy.
- Often data are presented as average consumption per day and this is less useful than data that describes portion size and frequency of consumption.
- There are a limited number of models to describe survival under chilling and freezing conditions.
- Specific consumption data are limited for most geographical locations.

Conclusions

The technical report illustrated that modelling exposure for *Salmonella* in broilers from "farm-to-fork" is a realistic proposition. The framework proposed in the report presented standard modelling techniques in a modular fashion and the output from such a framework could be readily integrated into risk characterization if required. Difficulties in modelling individual stages (e.g. limited data for pathway analysis) and the complexities associated with describing biological processes (e.g. cross-contamination) were identified.

The model framework illustrated the importance of suitable data inputs to ensure a robust exposure assessment. In particular, data should be representative, of appropriate quality and sufficient to meet the purpose and scope of the risk assessment.

Recommendations

The following recommendations for directing future work can be made.

- Reporting prevalence at different steps of the full exposure pathway should be encouraged in all regions of the world.

- Reported data should give full details of study methodology, including sampling site, sampling time, the relationship of the sample to the overall population and microbiological methods.

- Determination of quantitative data should be encouraged. If it becomes available then full exposure assessments could be developed to investigate mitigation strategies (e.g. use of chlorine in chill water) or compare alternative practices (e.g. air chilling versus immersion chilling).

- Cross-contamination during processing and handling operations should be studied quantitatively and methodologies for modelling this process should be developed. Cross-contamination during these stages is a critical factor that is often associated with outbreaks.

- The collection of consumption data should be promoted at the national level. The design of these studies should accommodate the data requirements for exposure assessments. These requirements include population variability, portion size and frequency of consumption.

- In predictive microbiology, survival has been less well studied than growth or death. There are few predictive models that describe survival at chill and frozen temperatures. Further development of these models is essential.

5.3.B Summary of discussion related to exposure assessment of *Salmonella* spp. in broilers

General Discussion

The consultation welcomed the technical report prepared by the expert drafting group as a significant contribution to the exposure assessment of *Salmonella* spp. in broilers. Although the structure of the model was strongly supported, any exposure assessment completed would be of limited representativeness because most input data was only obtainable from a small number of countries.

Different processing techniques, including freezing of chicken meat and carcasses, are common in the international trade of broiler products. Consequently, the effects of freezing on the concentration of *Salmonella* were identified as an

important data gap for exposure assessments addressing the international trade of poultry.

The aim of an exposure assessment is to model the dose of *Salmonella* consumed. When chicken products enter the kitchen they are subjected to a variety of preparation steps that introduce a wide range of opportunities for cross-contamination. Because it is difficult to identify and evaluate all of these processes, modelling of events in the kitchen is a difficult proposition.

The identification and acquisition of all potentially available data is a significant problem in conducting exposure assessments. In many cases the most desirable data for modelling is proprietary or unpublished. Commercial interests need some assurance that providing their proprietary data will not prejudice their business.

The consultation stressed the importance of clear tabulation of collected data with respect to ideal data requirements. In particular future presentation of such data should include, where possible, details of microbiological methods.

It was noted that specific sampling and enrichment methods used in studies influences the reliability and accuracy of the data, e.g. poultry rinse samples, swab samples and excised skin do not yield comparable results. Similarly, the culture of poultry litter samples may have a different sensitivity when compared with the culture of cloacal swabs.

Gaps in the data

- Information on the distribution of time and temperature for storage and cooking in a variety of national environments is lacking. Similarly pathways for cross-contamination are difficult to identify and model.

- Different enumeration methods vary in sensitivity. More sensitive enumeration methods are needed.

- More detailed data are required describing consumption patterns to improve risk estimates.

- Knowledge of survival times of *Salmonella* below 4ºC is essential to conduct predictive microbiological exposure assessments that are representative of international trade of poultry products.

- More data on *Salmonella*-free feed, *Salmonella*-free replacement stock, fasting prior to slaughter, and scalding, defeathering and evisceration processes are needed to effectively model the benefits of control interventions at the levels of production and processing.

5.4. ISSUES TO BE BROUGHT TO THE ATTENTION OF FAO AND WHO

- FAO/WHO should provide a means for the food and agriculture industry to provide proprietary data on pathogens and process variables in foods.

- FAO/WHO should request the CAC to provide focused risk management questions for further development of risk assessments on *Salmonella*.

6. Hazard characterization and exposure assessment of *Listeria monocytogenes* in ready-to-eat foods (RTE).

The *L. monocytogens* hazard identification, hazard characterization and exposure assessment technical documents presented to the consultation were discussed in detail by working groups. The full documents are available on request from FAO or WHO and can be found at the following internet addresses:

http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/pagerisk/riskpage.htm and http://www.who.int/fsf/mbriskassess/index.htm

The executive summaries of these documents were updated during the consultation to take into account some of the questions and comments on the papers resulting from these discussions and are presented below. These are followed by a summary of the discussions of additional points that were not directly incorporated into the executive summaries of the discussion papers.

Introduction

RTE foods, per definition, are not cooked or submitted to other listericidal treatments immediately prior to consumption. Consequently, occurrence and possible growth of *L. monocytogenes* in these products can lead to human exposure. The overall objective of the hazard characterization and exposure assessment for *L. monocytogenes* in different RTE foods is to obtain estimates of the health risk for groups of consumers or the population as a whole. These risk estimates can then be used for identification strategies and actions that decrease the level of that exposure risk. Exposure assessment and hazard characterization of *L. monocytogenes* in different RTE food relay on the virulence characteristics and microbial ecology in particular products. The documents discussed in the consultation and the summaries-discussions presented below includes several examples of how hazard characterization and exposure assessment of *L. monocytogenes* in RTE foods can be carried out.

6.1 HAZARD IDENTIFICATION AND HAZARD CHARACTERIZATION OF L. MONOCYTOGENES IN READY-TO-EAT FOODS

6.1.A Executive summary

Introduction

L. monocytogenes is widely distributed in the environment and has been isolated from a variety of sources including soil, vegetation, silage, faecal material, sewage and water. There is evidence to suggest that it is a transitory resident of the intestinal tract in humans, with 2 to 10 % of the general population being carriers of the organism without any apparent adverse consequences. The bacterium can grow at refrigerator temperatures and is resistant to various environmental conditions, allowing it to survive longer under adverse conditions than most other non-spore

forming bacteria. Most cases of human listeriosis are sporadic and the source and route of infection is usually unknown, however, contaminated food is considered to be the principal route of transmission. Foods most often associated with human listeriosis are ready-to-eat products that support growth of *L. monocytogenes*, have long refrigerated shelf lives, and are consumed without further listericidal treatments. Invasive listeriosis (i.e., severe *L. monocytogenes* infections) is a relatively rare but often severe disease with incidence rates typically of about 4 to 8 cases per 1,000,000 individuals and fatality rates of 20 to 30 % among hospitalised patients.

L. monocytogenes causes illness by penetrating the lining of the gastrointestinal tract and then infecting normally sterile sites within the body. The likelihood that *L. monocytogenes* will invade the intestinal tissue depends upon a number of factors, including the number of organisms consumed, host susceptibility, and virulence of the specific bacterial isolate ingested. All strains of *L. monocytogenes* appear to be pathogenic but their virulence, as defined in animal studies, varies substantially. Listeriosis is an opportunistic infection that most often affects those with severe underlying disease (e.g. immuno-suppressive therapy, AIDS, and chronic conditions such as cirrhosis that impair the immune system), pregnant women, unborn or newly delivered infants and the elderly. The bacterium most often affects the pregnant uterus, the central nervous system or the bloodstream, and manifestations of listeriosis include but are not limited to bacteremia, meningitis, encephalitis, endocarditis, meningoencephalitis, miscarriage, neonatal disease, premature birth, prodromal illness in pregnant women, septicemia, and stillbirth. Incubation periods prior to individuals becoming symptomatic can be from a few days up to three months.

L. monocytogenes can also cause mild febrile gastroenteritis in otherwise healthy individuals. The public health significance of this type of listeriosis is much lower than that of invasive listeriosis.

Objectives

The scope and objectives of the present work were to quantitatively evaluate the nature of the adverse health effects associated with *L. monocytogenes* in ready-to-eat foods, and to assess the relationship between the magnitude of foodborne exposure (the dose) and the frequency of these health effects (the response).

Approach

The approach taken by the expert drafting group was to review and to summarize the literature relevant to hazard characterization for this pathogen, and the available dose-response models. In the absence of human feeding studies and surrogate pathogens, a number of dose-response models based on epidemiological data, animal studies, expert elicitation or combinations of these are compared and evaluated. The dose-response relationships model the probability of different biological end-points such as infection, morbidity, or mortality, as a function of the ingested dose of *L. monocytogenes*.

Key findings

The issue of what functional form of the dose-response relationship best describes the reality of the interaction between *L. monocytogenes* and humans is not resolved. However, the highly variable response to exposure to a foodborne pathogen of a human population, indicates that the likelihood that any individual will become ill due to an exposure to a foodborne pathogen is dependent on the integration of host, pathogen, and food matrix effects. Several empirical relationships encompassing a variety of assumptions have been applied to modelling *L. monocytogenes* dose-response relations. These models may fit the data equally well but give widely differing predictions in the dose region corresponding to levels of *L. monocytogenes* commonly found in food. The influence of host factors has been addressed by developing relationships specifically for susceptible or non-susceptible individuals. The potential effects of the food matrix on the dose-response relation were not considered as a variable within any of the models due to insufficient data.

Available models, which to a varying degree and sophistication have been evaluated against human epidemiological data, include (categorised by the end-point being modelled): 1) Infection (Weibull-Gamma model, Exponential model, and beta-Poisson model); 2) Morbidity (Exponential model, USFDA/USDA model); 3) Mortality (USFDA/USDA model, Exponential model). All the models have assumed that, in theory, a single bacterial cell has the potential of causing disease. In experimental models this probability is expressed by the "r value" (see Table 6.1). Each of the dose-response models reviewed has specific characteristics and limitations (Figure 6.1 and Table 6.1). This figure is included for illustrative purposes and caution should be used in interpreting these curves since they are based on different endpoints, types of data etc., and in general, the predictions based on the models show a high degree of uncertainty and variation.

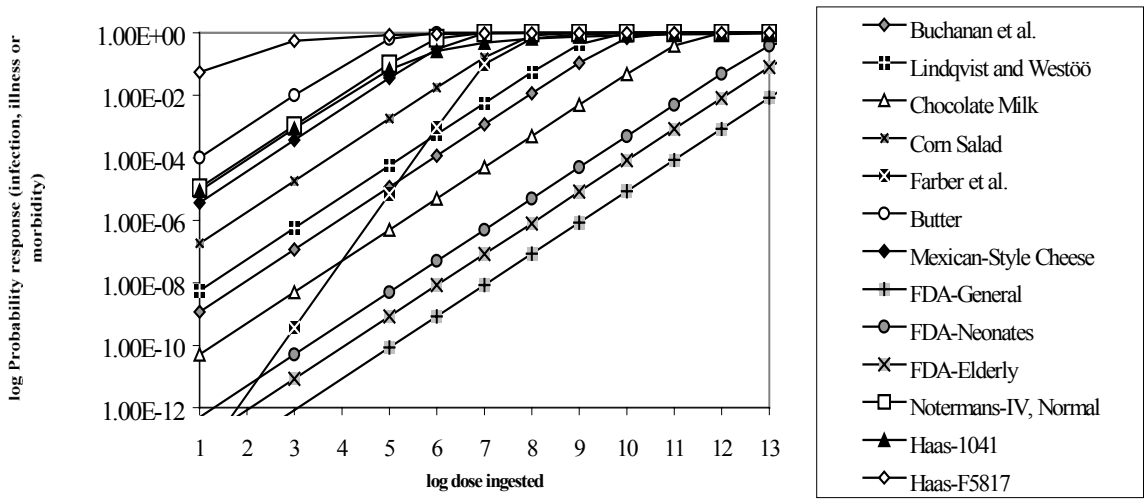

Figure 6.1. A comparison of available dose-response models for describing infection, morbidity or mortality.
NOTE: The points on the curves are only for legend purposes and do not represent data points.

Table 6.1. Summary of the selected dose-response models available for *Listeria monocytogenes* that were reviewed in the current document. The models are summarised in the order they are shown in the legend of Figure 6.1.

Model/Study	Biological End Point	Model/ Parameters	Comments
Buchanan *et al.* (1997)	Morbidity (serious listeriosis) Based on annual statistics and food survey data	[1]Exponential *r = 1.18×10^{-10}	Based on an estimate of immunocompromised individuals. It is purposefully conservative and assumed that all cases were caused by a single food category. Predicted $Morbidity_{50} = 5.9 \times 10^{9}$ CFU. r-value is approximately equal to the probability that a single cell of *L. monocytogenes* could cause serious illness.
Lindqvist and Westoo (2000)	Morbidity (serious listeriosis) Based on annual disease statistics and food survey data	[1]Exponential *r = 5.6×10^{-10}	Based on an estimate of immunocompromised individuals. It is purposefully conservative and assumed that all cases were caused by a single food category. Predicted $Morbidity_{50} = 1.2 \times 10^{9}$ CFU. r-value is approximately to the probability that a single cell of *L. monocytogenes* could cause serious illness.
Chocolate milk, Current Study	Febrile gastroenteritis	[1]Exponential *r = 5.8×10^{-12}	Based on an outbreak associated with consumption of chocolate milk. The population was limited to immunocompetent individuals who suffered gastroenteritis symptoms only.
Corn salad, Current Study	Febrile gastroenteritis	[1]Exponential *r = 1.8×10^{-8}	Based on an outbreak associated with consumption of corn salad. The population was limited to immunocompetent individuals who suffered gastroenteritis symptoms only. The dose response curve may be highly conservative due to highly limited dose data.
Farber *et al.* (1996)	Serious infection in humans. Based on expert elicitation	[2]Weibull-Gamma, $\alpha = 0.25$ $\beta_{High\ Risk} = 10^{11}$ $b = 2.14$	The dose estimated for 50% of the population to become infected: High Risk: 480,000 CFU Low Risk: 48,000,000 CFU The model is of limited usefulness due to over prediction of the number of serious illnesses and a general lack of transparency regarding how the various assumptions were reached.
Butter, Current study and FDA (2000)	Morbidity (Serious listeriosis) Analysis of outbreak data	[1]Exponential *r = 1.02×10^{-5}	Based on an outbreak in Finland caused by butter. The effected population was a group of highly immunocompromised individuals in a hospital setting. Predicted $Morbidity_{50} = 6.8 \times 10^{4}$ CFU
Mexican-style cheese, Current study and FDA (2000)	Morbidity (Perinatal listeriosis) Analysis of outbreak data	[1]Exponential *r = 3.7×10^{-7}	Based on an outbreak in pregnant women in the United States caused by Mexican-style cheese. Predicted $Morbidity_{50} = 1.9 \times 10^{6}$ CFU.
FDA-General, FDA (2000)	Mortality Based on combination of animal (mice) lethality and human fatality statistics	[3]Original model based on weighted, multiple mathematical models. The current study used an exponential model in conjunction with predictions for the 10^{12} dose to represent *r = 8.5×10^{-16}	Model includes individuals between the ages of 30 days and 60 years. It includes consideration of distributions for strain virulence. It is based on mouse lethality data "anchored" so that the model provides prediction consistent with incidence of lethal *L. monocytogenes* infections reported in FoodNet (The US Foodborne Diseases Active Surveillance Network).

* r = probability of a single cell causing infection
[1] Exponential model: single parameter. Microorganisms are assumed to occur randomly and independent
[2] Weibull-Gamma model: Three parameters. Based on Weibull model, host/pathogen interaction follows a distribution modified by two factors.
[3] US FDA/USDA model: Surrogate experimental animal is used to establish the shape of the dose-response curve. US epidemiological data used to set constraint limits (anchor the model)

			The number of cases of serious listeriosis was estimated by multiplying predicted fatalities by a factor of 5. The LD_{50} associated with this r-value should be considered notional and interpreted as indicating that a significant portion of the population is not susceptible.
FDA-Neonates, FDA (2000)	Mortality Based on combination of animal (mice) lethality and human fatality statistics	[3]Original model based on weighted, multiple mathematical models. The current study used an exponential model in conjunction with predictions for the 10^{12} dose to represent *r = 5.0×10^{-14}	The model includes foetuses and neonates less than 30 days of age. It assumed that exposure is *in utero*. It includes consideration of distributions for strain virulence. It is based on mouse lethality data and "anchored" so that the model provides a prediction consistent with incidence of lethal *L. monocytogenes* infections reported in CDC FoodNet. The LD_{50} associated with this r-value should be considered notional and interpreted as indicating that a significant portion of the population is not susceptible.
FDA-Elderly, FDA (2000)	Mortality Based on combination of animal (mice) lethality and human fatality statistics	[3]Original model based on weighted, multiple mathematical models. Current study used exponential model in conjunction with predictions for the 10^{12} dose to represent *r = 8.4×10^{-15}	The model includes individuals over 60 years of age and consideration of distributions for strain virulence. Based on mouse lethality data and "anchored" so that the model provides prediction consistent with incidence of lethal *L. monocytogenes* infections reported in FoodNet. The number of cases of serious listeriosis is estimated by multiplying predicted fatalities by a factor of 5. The LD_{50} associated with this r-value should be considered notional and interpreted as indicating that a significant portion of the population is not susceptible.
Notermans-IV, normal, Notermans *et al*., (1998)	Mortality in mice	[1]Exponential Model *r = 1.1×10^{-6}	It is based on mice injected IV with *L monocytogenes*. Mice were previously exposed to *L. monocytogenes*. Mice that were not previously exposed were more susceptible to *L. monocytogenes*. The use of mortality in mice without correction for the apparent decreased susceptibility of humans for *L monocytogenes* led to a substantial overestimation of mortality in humans
Haas *et al*. (1999)	Infection in mice	[4]Beta-Poisson and Exponential (no fit) <u>Strain 1041</u> $\alpha = 0.17$ $ID_{50} = 2.1 \times 10^{6}$ <u>Strain F5817</u> $\alpha = 0.25$ $ID_{50} = 2.8 \times 10^{2}$	Using infection in mice without correction for the apparent decreased susceptibility of humans for *L. monocytogenes* led to a substantial overestimation of the incidence of infection in humans. The selection of the end point of infection of normally sterile sites in mice is difficult to correlate with human disease.

* r = probability of a single cell causing infection
[1] Exponential model: single parameter. Microorganisms are assumed to occur randomly and independent
[3] US FDA/USDA model: Surrogate experimental animal is used to establish the shape of the dose-response curve. US epidemiological data used to set constraint limits (anchor the results)
[4] Beta-Poisson model: Two parameters. Heterogeneity of host/pathogen interaction

At this stage it is not possible to endorse a single dose-response model. In part, this reflects the fact that the models are based on different biological end points and use different types of data (e.g. annual disease statistics, animal models, outbreak investigations)(Table 6.1). The use of several dose-response model relationships is the recommended approach to deal with the uncertainty related to our current gaps in knowledge of dose-response relationships. Presently, there are only limited criteria on which to base the selection of a dose-response model and better ways to evaluate the models are needed. However, the choice of which models to use will depend on factors such as the purpose of the risk assessment and the level of resources and sophistication available to the risk assessors. This requires that the basis for the various dose-response relations and their impact on the overall risk assessment be adequately communicated to the risk managers requesting the assessment.

The absence of human data, the incomplete epidemiological information, the difficulties in extrapolating from animal data to humans, and a lack of mechanistic models are all limiting factors identified here that contribute to the uncertainty in the description of the dose-response relationship. The approach taken in the USFDA/USDA model is noteworthy since it addresses several of these limitations but it will need further evaluation and it would be interesting to evaluate that model with additional and independent data that has not been used to calibrate the model.

Gaps in the data

The limitations of the dose-response models reviewed, reflects the need for further data and scientific understanding of the pathogen's mechanisms of pathogenicity and the host and food matrix effects that influence the potential to cause disease. Priority knowledge gaps that were identified in this evaluation include:

- Impact of food type (food matrix) on the ability of *L. monocytogenes* to cause disease.

- Identification of key virulence factor(s) within *L. monocytogenes* isolates that lead to the apparent diversity in the ability of strains of this pathogen to cause disease.

- Determination of the distribution of virulence potentials among *L. monocytogenes* isolated from foods.

- Enhanced epidemiological data related to outbreaks and sporadic cases of listeriosis, particularly data needed to calculate attack rates, to determine the dose consumed by individuals, and to assess the health and immune status of both symptomatic and asymptomatic individuals.

- Better estimates of the actual proportion of the population at increased risk of invasive listeriosis.

Conclusions

While there are limitations associated with each of the dose-response models for *L. monocytogenes* evaluated in this report, it can be concluded that there are several models that could be useful in developing risk assessments for this

microorganism. However, at the current time it would be advisable to consider the use of multiple dose response models when estimating risks. Higher consideration should be given by users of the models to those models that provide a more accurate picture of the dose-response relations by capturing the full interaction of host, pathogen, and food matrix effects.

Use of any of the models should be consistent with the fact that serious invasive listeriosis is a rare foodborne disease that largely, but not totally, affects specific high risk populations. However, even in these groups the likelihood of disease appears low. Thus, it is expected that dose-response models based on animal models that were selected for their susceptibility to *L. monocytogenes* would be of limited usefulness for predicting human response unless the dose-response relations for the animal model can be appropriately correlated to the disease response in humans. Without an appropriate basis in human disease, such models may not yield estimates of risk that are accurate and useful. Using animal data it appears that modelling lethality or severe invasive listeriosis is more effective in relation to human disease than modelling infection. At the current time, the public health significance of febrile gastroenteritis is largely unknown. The circumstances leading to these symptoms among the normal population appears sufficiently different so that the usefulness of using this as a biological end point for developing dose-response relations is limited.

It appears that dose-response relationships developed using epidemiological and annual health statistics from one country may be useful in predicting the dose-response relations in populations of other countries with a similar level of development. The same models may be applicable to other countries as well, but the influence of differences in demographics and the size of sub-populations at increased risk should be considered.

Recommendations

- Consider the use of multiple dose-response models when estimating risk.
- Develop criteria to form the basis for selecting dose-response models and tools to compare them.
- Evaluate the dose-response models by testing them against independent data.
- Avoid the use of febrile gastroenteritis as a biological end point for modelling.
- Evaluate the effect of the type of food (food matrix) on the ability of *L. monocytogenes* to cause disease.
- Identify key virulence factor(s) within *L. monocytogenes* isolates that lead to the apparent diversity in the ability of strains of this pathogen to cause disease.
- Determine the distribution of virulence potentials among *L. monocytogenes* isolated from foods.

- Obtain epidemiological data needed to calculate attack rates, determine the exposure dose, and assess the health and immune status of both symptomatic and asymptomatic individuals.

- Develop estimates of the high risk population.

6.1.B Summary of discussions on hazard identification and hazard characterization

Limitations of dose-response models for hazard characterization of L. monocytogenes

There is epidemiological data indicating that low doses of *L. monocytogenes* can cause listeriosis. Conversely, quantitative exposure assessments indicate that all consumers are exposed to very high doses of *L. monocytogenes* in RTE foods many times per year. This suggests a missing element in our understanding of foodborne listeriosis due to RTE foods that is possibly related to variability in virulence between strains of this microorganism. For example, the establishment of "epidemic" strains in processing environments is recognized as an element common to several outbreaks of listeriosis.

Virulence of L. monocytogenes

All strains of *L. monocytogenes* are currently considered virulent and no acceptable biomarker has been developed to detect virulence of strains and host factors that may relate to increased susceptibility.

Application of dose-response models to different countries

Dose-response models may be generic. However, models would probably gain in international applicability through use of input data from different regions of the world. Validation of models by using such data has been encouraged.

6.2. EXPOSURE ASSESSMENT OF L. MONOCYTOGENES IN READY-TO-EAT FOODS

6.2.A Executive summary

Introduction

The scope and objective of the present work were to quantitatively assess human exposure to *L. monocytogenes* in ready-to-eat (RTE) foods. The exposure assessment estimates the number of meals containing the pathogen and the number of organisms consumed. An exposure assessment can then be combined with a hazard characterisation, so that the magnitude and severity of risks to human health can be estimated.

Objectives

The aim of this report on exposure assessment of *L. monocytogenes* in RTE foods is to:

- Provide an overview of issues that should be considered.
- Describe and evaluate methods that can be used.
- Collate and present relevant data and information.
- Demonstrate the application of exposure assessment to specific risk management questions for use in both industrialised and developing countries.

Approach

The report considers issues related to assessment of exposure including an extensive review of general principles and modelling approaches, and provides a glossary of technical terms. Discussion of the merits of different approaches and their relevance to different risk questions is also presented.

Eleven examples of exposure assessments from existing qualitative/descriptive and quantitative risk assessments or related documents are reviewed and assessed using criteria based on the 1999 Codex principles and guidelines for the conduct of microbiological risk assessment. These show different approaches and ideas for modelling exposure to *L. monocytogenes* in specific RTE foods or in different countries or regions.

In addition seven new exposure assessments were initiated. The selection of these examples was based on various criteria, including:

- different food commodities
- lightly processed and highly processed RTE foods
- a history of listeriosis associated with the food
- potential for growth or inactivation during long-term storage
- potential affect of temperature abuse
- the effect of an inactivation step, e.g., pasteurization
- potential for post-processing contamination
- high consumption rates
- use in international trade

The following RTE foods were modelled from retail to the point of consumption.

- raw and unpasteurized milk
- ice cream
- soft mould-ripened cheese

The following RTE foods were modelled from production to the point of consumption:

- minimally processed vegetables
- smoked salmon

- semi-fermented meats

In addition, there is a specific example comparing the effect of zero tolerance and a tolerance of 100 CFU/g at the point of consumption.

The aim of these examples is to illustrate the effect on exposure of:

- processing
- low contamination levels in products that do not permit growth of *L. monocytogenes*
- long-term storage on increase or decrease of *L. monocytogenes* concentration
- consumption frequency and meal (serving) size.

Gaps in the data that currently prevent completion of exposure assessments are identified and recommendations given on improving exposure assessments and their use for RTE foods. To supplement the report, a substantial bibliographic list of sources of relevant information and data is appended, but references specific to the different sections are also included in the report.

Key Findings

The published and unpublished assessments that were reviewed included assessments of *L. monocytogenes* in bovine milk; seafoods; smoked salmon and trout; soft cheese made from raw milk; shredded cabbage; processed meats; and 21 RTE food groupings. These assessments were prepared in Australia, Canada, France, Sweden and the U.S. In addition, the report of an FAO expert consultation on the trade impact of *Listeria* in fish products was reviewed.

All assessments had clear goals, but these goals were quite different resulting in different approaches and levels of sophistication being adopted. All commented on the lack of data available and the consequential need to make a number of assumptions. The assumptions were incorporated into a modelling approach that was well developed in some examples, but less so in others, representing an evolution of sophistication over the six year period (1994 – 2000) since the first assessment of exposure to *L. monocytogenes* in RTE foods was published. No assessment critically considered the effect of the assumptions inherent in the model.

While all of the approaches that are recommended in the current report were used in one or other of the exposure assessments reviewed, none fully encompasses a "farm-to-fork" approach using a fully stochastic model. The most extensive study assessed exposure from many types of food grouped into 21

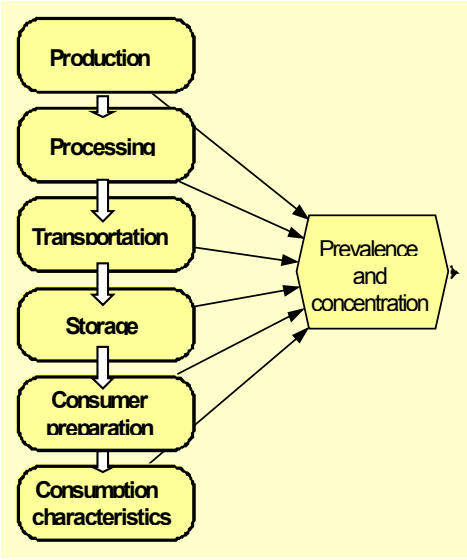

Figure 6.2: Product flow diagram indicating the tracking of the changes in prevalence and concentration of *L. monocytogenes* in a food.

categories with *L. monocytogenes* growth modelled only from retail to consumption.

For the novel example exposure assessments a generic model structure was developed and is shown in Figure 6.2. It illustrates the need to track changes in the prevalence and concentration of *L. monocytogenes* in RTE foods as they move through the food system to the point of consumption. Figure 6.3 illustrates the interaction of factors that influence the level of exposure and the need to differentiate exposure to different population subgroups. In a completed exposure assessment the typical result is the simulated number of *L. monocytogenes* in a contaminated serving. For instance, in the model for exposure from soft cheese, the predicted distribution of *L. monocytogenes* concentration in servings is shown in Figure 6.4. The exposure assessment further predicts that for consumers at normal risk, from 4 to 22 servings of soft cheese are consumed per year, and that for consumers at high risk, from 3 to 17 servings of soft cheese are consumed per year. Of those soft cheese servings, 4 % (median) are predicted to be contaminated (Figure 6.4).

The starting point in the exposure assessment depends on the question that the risk manager seeks advice on. For example, in the case of RTE foods that do not receive a listericidal step, it may be necessary for the assessment to include potential sources of contamination in the harvest or growing area. Alternatively, for those products that do undergo a listericidal step but may become contaminated or re-contaminated, only the steps subsequent to the listericidal step may need to be modelled.

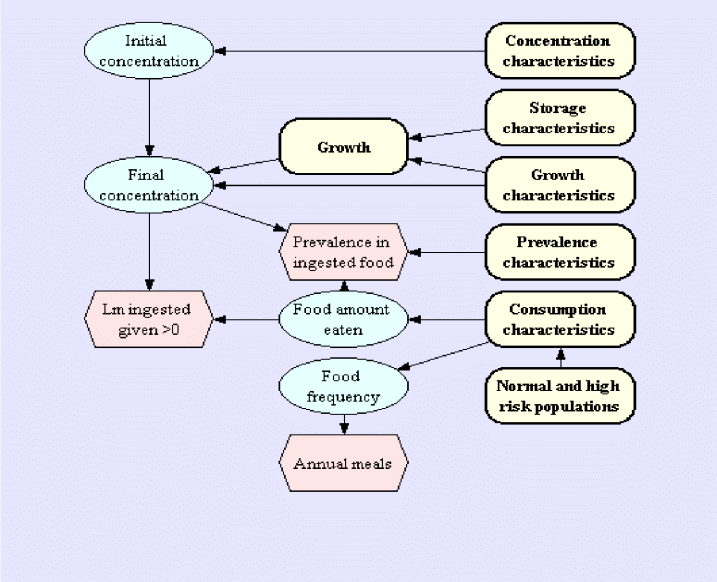

Figure 6.3. Influence diagram detailing factors that affect human exposure to *L. monocytogenes* in RTE food.

Food consumption characteristics of sub-groups of the population that are particularly susceptible to listeriosis need to be determined, but this proved difficult from available survey data.

An assessor often needs to be able to determine the prevalence and concentration at one point in the food chain from an earlier point in the chain because specific data relevant to the point of consumption is unavailable. This is also important in order to be able to assess the effectiveness of proposed intervention steps. To overcome lack of data, or to perform a process risk assessment, mathematical modelling will normally have to be used and the appropriate assumptions made. Growth, survival and death models for *L. monocytogenes* are now available, including models that reasonably predict growth rate in pure cultures and challenge tests. However, there is evidence that models may be less accurate for foods naturally contaminated with *L. monocytogenes.* Exposure assessments must explicitly

recognize the limitations of the current generation of predictive microbiology models so that the risk assessment process is transparent.

While the stochastic modelling approaches were found to be preferable, there are potential disadvantages. For example, as the complexity of the model increases, the bounds of uncertainty/variability become wider and may become so broad as to convey little useful information to the risk manager.

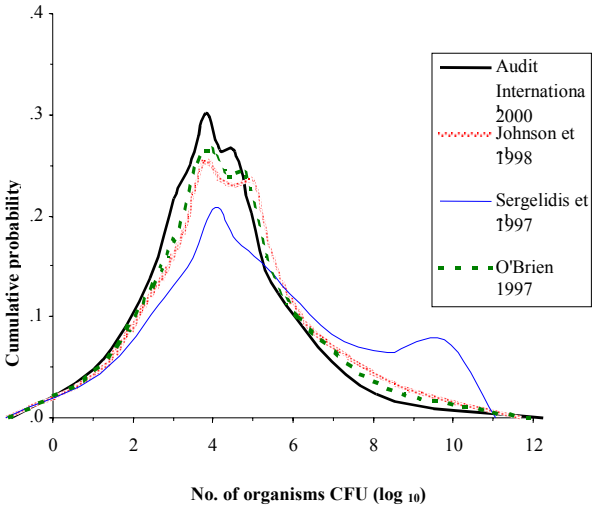

Figure 6.4: Simulated distribution of number of *L. monocytogenes* organisms in those 4% (median) of servings that are predicted to be contaminated. The 4 lines represent the outcome based on 4 different studies of home refrigerator storage conditions.

Useful insights can be obtained by using a risk assessment approach. However, the full range and quality of data required to complete risk assessments is not currently available. Specific gaps that were identified in the data are listed separately below.

Gaps in the data

- *L. monocytogenes* incidence/prevalence in potential environmental sources, including i) agricultural environments, e.g. ground and well water; cultivated soil used for different crops or uncultivated soil for grazing pasture at different times of the year, silage, fresh and composted manure, farm equipment and farm workers; ii) aquatic environments, marine and freshwater where fish or shellfish are harvested including the effects of sewage or agricultural runoffs into water, fishing equipment, and commercial and recreational fishers.

- *L. monocytogenes* incidence/prevalence and concentration in production including i) primary production: animals, fish, and crops; ii) secondary production, e.g. initial preparation, cleaned carcasses, gutted and stored fish, shucked shellfish, washed produce.

- Product formulation information, e.g. pH, water activity and humectants, preservatives (e.g. nitrite and organic acids, lactic acid bacteria) to enable best estimates of microbial growth, survival or death.

- Data for evaluating the validity of predictive models for *L. monocytogenes* in specific products, recognising the effect of prior history of the culture and potential differences between naturally contaminated products and those deliberately inoculated in challenge tests.

- Identification of virulence markers for *L. monocytogenes* is needed so that exposure to those strains can be assessed specifically.

- Identification of sources and levels of contamination and recontamination, both at the point of processing and at retail are needed, with information on frequency, and microbial load transferred.

- Impact of microbiota including spoilage organisms on the growth and survival of *L. monocytogenes* in RTE foods or their ingredients, and on the shelf life of products.

- Prevalence and concentration of *L. monocytogenes* on finished packages of RTE foods.

- Retail and consumer handling practices, in particular, storage time and temperature and including more accurate measurements of home storage conditions including refrigeration temperatures by country or region.

- Specific RTE product consumption data for meal servings and frequency by specific populations of individuals, including in developing countries, and particularly for those who are immunocompromised or otherwise susceptible.

- Epidemiological data that distinguish serious or life threatening (usually systemic) vs. mild disease.

Conclusions

The report has demonstrated that it is feasible to develop exposure assessments useful to those managing food safety risks but that there are significant gaps in the data required to complete assessments for *L. moncytogenes* in RTE foods. For example, in the short term it will be necessary to characterize consumption frequency and meal size, and to obtain information on home storage times and temperatures, and handling and preparation practices.

Despite these limitations, immediate benefits are attainable using model exposure assessments by combining them with hazard characterizations to complete the risk characterization. As long as there are insufficient data, exposure assessment will remain reliant on the use of models of food production and distribution systems, of microbial ecology in foods, and on optimising the use of existing data. Specifically, to facilitate the development of exposure assessment by both experienced and inexperienced assessors, it is recommended that guidelines be developed and made widely accessible for the pooling of data from difference sources and of uncertain compatibility and quality. Also guidelines on the appropriate use and development of models, including testing of model validity, assessment of the effect of assumptions, and communication of the reliability (e.g. confidence intervals) of model results should be developed.

To maximise the use of models, they should be developed and described in a transparent manner so that they can be adapted and modified to the changing needs of the risk managers or adopted and adapted by other risk managers.

In the long term, the report identified the need for the establishment of an international data repository for exposure assessment data, including data from the

food industry. The need to establish food-borne disease surveillance systems was also recognized.

Recommendations

Exposure assessments require data on prevalence (frequency of contamination and eating) and dose (contamination level and meal sizes for all ages including children and the elderly). To address some of the critical gaps in the data it is recommended that:

- Member Countries be encouraged to identify and report on local practices of storage and handling of food in the home by consumers and in food-service establishments, including storage temperatures and times.

- Coordination be sought with nutrition/consumption studies to develop knowledge of consumption data relevant to foodborne microbiological risks.

- A central, international, data repository be established so that, once collated, data can be accessed and accumulated for use in future exposure assessments.

- Systems of data input are developed whereby industry can contribute their data to the exposure assessment processes without fear of prejudicial use of their data including punitive action by government or release to competitive industries.

- Active surveillance programs are established to develop information against which exposure assessments can be validated.

To advance the development of rigorous and reliable exposure assessments it is recommended that:

- A process is established to indicate the types of exposure assessments that can be used by experienced assessors and those who are new to the process, as well as an approach to modify the model assessments to answer the types of questions that risk managers ask.

- All assumptions used in the development of exposure assessment models should be stated explicitly, and where there are assumptions, the validity of those assumptions is tested and confidence in derived estimates of exposure be specified or characterised. The reliability (e.g. confidence intervals) of model results should be communicated.

- Mathematical modelling guidelines be established for use in microbial food safety exposure assessment. An international workshop, such as that conducted for hazard characterization, may be an appropriate mechanism. This would include guidelines for predictive microbiology aspects of exposure assessment, pooling of data from difference sources and of uncertain compatibility and quality and data editing.

6.2 B Summary of discussions on Exposure Assessment

Comprehensiveness and costs of exposure assessments.

Development of an exposure assessment can be costly, particularly when the generation of new data is required, for example, for occurrence of growth of *L. monocytogenes*. The comprehensiveness and thereby the costs of an exposure assessment must reflect the importance of the problem under study.

Variability in the prevalence of L. monocytogenes in foods from similar processing plants/production lines.

There is evidence that at least for some RTE foods from similar processing plants/production lines the prevalence of *L. monocytogenes* in the products can vary from non-detectable to 100%. If quantified, this variability in prevalence of *L. monocytogenes* can be taken into account in exposure assessments. However, the factors resulting in this variability often remain unknown and these need to be identified to evaluate the potential and effect of lowering the prevalence of *L. monocytogenes.*

Collection of data on foodborne listeriosis

Collection of data from outbreaks is one possible way to increase knowledge about exposure to doses of *L. monocytogenes* from RTE foods. This type of data collection could be improved where there are guidelines provided by governments for outbreak investigations. However, collection of exposure data from outbreaks is difficult due to the long incubation period of listeriosis and the unavailability of the exposure food source at the time of illness. Co-ordination between food and health authorities to provide data relevant to risk assessment has rarely been effective. It is also recognized that large outbreaks of food-borne listeriosis are the exception. It appears that there are many sporadic cases of listeriosis that are not investigated or their food association is not determined.

Socio-economic factors

The relationship between the exposure to the microorganism and factors related to socio-economic status, food preparation and storage practices, food consumption patterns, frequency of consumption and other related aspects specific to RTE foods needs further research.

6.3 ISSUES TO BE BROUGHT TO THE ATTENTION OF FAO AND WHO

Gathering of information:

- There is a need for collection of data related to all aspects of exposure assessment and hazard characterization. Some of the data needed is likely to be available, for example, within private industries and national or regional food inspection services. It is suggested that future efforts for data collection should also be targeted directly to these potential providers of specific information.

- There is a general lack of quantitative data for use in exposure assessment, for example, data for levels of contamination as well as prevalence, growth or inactivation kinetics in RTE foods.
- There is a need for information on consumption frequency and meal size, as well as data on home storage times and temperatures, handling and preparation practices.
- There is also need for information on *L. monocytogenes* disease incidence, predisposing conditions, clinical manifestations (invasive vs. gastrointestinal) and probable exposure sources, giving consideration to regional differences.
- It is further suggested that the potential for setting up an international data repository should be investigated.

Research:

- Research is needed on virulence factors to better understand the diversity of *L. monocytogenes* virulence and to identify markers to differentiate between strains.
- Increased understanding about the ecology of *L. monocytogenes* is needed to enable identification of routes of contamination and thereby reduce prevalence and growth in RTE foods.
- Development of sensitive methods for enumeration of *L. monocytogenes* is needed to determine and quantify presence and growth of low levels (< 100/g) of the organism in RTE foods and environmental samples.
- Collaboration is needed to explore new approaches to develop dose-response models and stimulate efforts to test the credibility of existing methods with independent data.
- Criteria to form the basis for selecting dose-response models and tools to compare them need to be developed.
- Potential intervention strategies to reduce the prevalence and concentration of *L. monocytogenes* in RTE foods should be explored through the stochastic modelling of exposure routes.

Technical support for developing countries

- Sustainable support for training in the form of targeted, dedicated courses is required to assist the transfer of technology for microbiological risk assessment to developing countries.

7. Guidelines on Hazard Characterization

7.1. INTRODUCTION/CURRENT STATUS

The process for the development of guidelines on hazard characterization for microbiological hazards in food and water began at a workshop held in Bilthoven, the Netherlands, 13-17 June 2000. The objective of the Hazard Characterization Workshop was to develop guidelines on hazard characterization for microbiological hazards in food and water. In order to accomplish this, the workshop 1) reviewed the state of the art in hazard characterization and relevant scientific disciplines (e.g. epidemiology, biomedical research, mathematical modelling); 2) categorized the principles and methods of hazard characterization; 3) provided guidance on the type of data needed and the means of assessing the adequacy of available data for developing dose-response relationships for specific pathogens; and 4) identified future research requirements to reduce uncertainty of dose response models and default assumptions for use in the short term.

Participants at the Bilthoven workshop compared and reviewed the approaches used in hazard characterizations for several pathogens (*Salmonella spp.*, *L. monocytogenes*, enterohaemorrhagic *Escherichia coli*, *Cryptosporidium parvum* and Norwalk-like viruses). The outcomes of the workshop included a comparison of models that illustrated the benefits and weaknesses of different approaches. The workshop formulated general principles and guidelines for hazard characterization that were further discussed at this expert consultation.

The consultation discussed and reviewed the working principles and guidelines developed at Bilthoven with the intention of further developing the document in a wider arena of experts. In addition, practical lessons from the specific hazard characterization documents prepared by the expert drafting groups were included. The guidelines, in their current status, are a work in progress and will receive a variety of inputs in the medium term before completion.

7.2. SUMMARY OF THE DISCUSSIONS

The consultation welcomed the opportunity to participate in the continued development of these guidelines. General discussions indicated wide support for the purpose and scope of the work. Areas for further technical developments were specifically identified and comments on format, scope and structure are included in this summary.

General issues

- The document should clearly and consistently state that the purpose and scope of a hazard characterization depends on the purpose and scope of the specific risk assessment that is required. This will generally be determined by risk managers in association with risk assessors and must facilitate achievement of identified risk management goals.

- Hazard characterization of microbiological hazards will inevitably include consideration of biological characteristics of the microorganism, food matrix, and human host and their impacts on adverse health effects. This extends the

understanding of hazard characterization as currently defined in the Codex Alimentarius: ("The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard. For the purpose of microbiological risk assessment (MRA) the concerns relate to microorganisms and or their toxins." (CAC/GL- 30 1999)). Thus, hazard characterization for microbiological hazards in foods must be extended to cover all components of the pathogen, food, and host triangle.

- Consideration of decisions on tolerable levels of risk are risk management functions and, therefore, not within the scope of the Guidelines Document.

- Opinion was expressed that the working document is too closely linked to experience gained from hazard characterizations contained in relatively few pathogen / food commodity examples. Consideration of other approaches should be added.

Specific issues in the current document

- The introduction of the document should clearly describe the way in which hazard characterization contributes to the process of risk assessment.

- The introduction contains considerable repetition, which should be addressed in the next revision. Figure 1 should be presented in a narrative format.

- In section 5.1.1.3, the orientation of the hazard characterization will be a consequence of the specific purpose of the risk assessment to which it contributes.

- The representativeness and adequacy of data included in model inputs are major issues in MRA. Section 6 should be expanded to include further development of guidelines on these topics. Further to this work, the issue of "qualitative" risk characterisations remains controversial and will require further deliberation. Section 6 should also include strategies to acquire high-quality data.

- Characterization of human adverse health effects (Section 7.1) should include reference to methods other than standard dose-response modelling. In particular the use of epidemiological methods (such as case-control, cohort or cross-sectional studies) should be emphasized in the guidelines. For instance, a comprehensive set of epidemiological studies could be used to elucidate the relative importance of various food types for a specific hazard. Establishing a dose-response function should be based on the consideration of an array of different mathematical models, as appropriate.

- Information on the extent and severity of disease that is included in dose-response modelling (Section 7.1.2) should be appropriate to the purpose and scope of the particular risk assessment (e.g., differences in socio-economic and demographic characteristics of consumer populations in different countries).

- The selection of models in Section 7.2.2 should not advocate building "conservativeness" into the model structure. This concept applies to treatment of inputs and outputs rather than the structure of the model of itself, which should be precise and unbiased.

- The Bilthoven workshop rejected the idea of a threshold in infection for microbial hazards, as the weight of current evidence suggests that one ingested organism has some probability (however remote) of causing illness. Further debate on this issue may lead to the removal of any consideration of thresholds from the working document.

7.3. FUTURE WORK

The expert consultation concluded that the working principles and guidelines developed at Bilthoven should be the subject of further review and development to incorporate the changes suggested above. The opinion of the Codex Committee on Food Hygiene should be solicited together with ongoing input for the Joint Expert Consultations on Microbiological Risk Assessment. In addition, comments will be solicited from the general public through publication on the WHO and FAO web sites. Development of the guidelines will be finalized at a second Joint Expert Consultation on Microbiological Risk Assessment in 2001.

8. Conclusions of the expert consultation

The consultation recognized the comprehensiveness and scientific value of the technical reports presented and concluded that they considerably advanced current knowledge on the general development of hazard characterization and exposure assessments. These reports also enhanced specific knowledge in relation to the pathogen-commodity combinations identified as significant food safety problems by the CCFH. In the absence of specific risk management guidance, the consultation endorsed the approach taken by the expert drafting groups in developing hazard characterizations and exposure assessments for *L. monocytogenes* in ready to eat foods and *Salmonella* spp. in broilers and eggs, although they were not tailored to achieve specified risk management goals. This practical approach advances international understanding in a broad sense and provides a strong platform for future provision of risk assessment advice as requested by FAO/WHO Member Countries, the CCFH, and other stakeholders.

The consultation recognized that international scientific cooperation and peer review is essential to develop credible hazard characterization and exposure assessments for the purpose of risk characterization. The consultation agreed on the general applicability of modelling approaches taken and reflected on the critical importance of adequate data inputs to satisfy risk management goals. Additionally, it recognized technical areas where scientific consensus has not been achieved and ongoing scientific dialogue is necessary to resolve such issues. In development of individual components for the purpose of risk characterization of specific pathogen-commodity combinations as identified by the CCFH, the consultation stressed that the purpose and the scope for individual risk characterizations will influence data acquisition needs.

The consultation recognized the essential need for FAO/WHO to continue their support of these FAO/WHO Expert Consultations in relation to completing risk assessment of the pathogen-commodity combinations identified as significant food safety problems by the CCFH.

The consultation recognized the specific need for these risk assessments to be applicable to developing countries and the data requirements needed to achieve this. Likewise, it was recognized that a prerequisite for further development of microbiological risk assessment in developing countries is the provision of appropriate technical advice, assistance and training.

9. Recommendations

The consultation recommended that FAO and WHO should:

- Continue the ongoing work on developing guidelines for hazard characterization;
- Initiate a similar process for developing guidelines for exposure assessment;
- Continue to support the technical development of the hazard characterization and exposure assessment for the three pathogen-commodity combinations described in this report and identified by the CCFH as important candidates for risk assessment;
- Facilitate the acquisition of data for hazard characterization and exposure assessment for identified food safety priorities to the greatest extent possible;
- Promote the collection of consumption data at the national level. These requirements include population variability, portion size and frequency of consumption;
- Promote acquisition of model input data from different regions of the world in order to maximize the applicability of existing exposure models;
- Promote the establishment of regional centres for collection of information on disease incidence on a global scale so as to enhance the validation of risk assessment models;
- Develop a framework document for guiding the establishment of repositories for food safety data critical for effective risk assessment;
- Facilitate direct technical cooperation between developed and developing countries so that they achieve the technical capability required to do microbiological risk assessment. This support should take into consideration the local situation in order for the result to be sustainable.
- Explore ways to further evaluate the importance of [*]food saving systems that have recently been recognized as useful as a means of obtaining quantitative data in the case of outbreaks.

[*] Food saving systems: A system whereby all large foodservice establishments are advised to keep frozen portions of prepared foods for a specified time period for subsequent testing in the case of illness being associated with the food.

In addition the consultation recommended that:

- Any requests by risk managers for the development of hazard characterization or exposure assessment should include a clear description of purpose and scope.
- Reporting of prevalence and concentration of specified hazards at different steps of the full exposure pathway should be encouraged in all regions of the world.
- FAO and WHO are encouraged to assist developing countries in the preparation of project proposals on microbiological risk assessment activities for presentation to potential donors.

Annex 1: Participants

EXPERTS

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Listeria monocytogenes in ready-to-eat foods

Hazard Characterization

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Exposure Assessment

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Salmonella spp. in broilers and eggs

Hazard Characterization

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Exposure Assessment – Eggs

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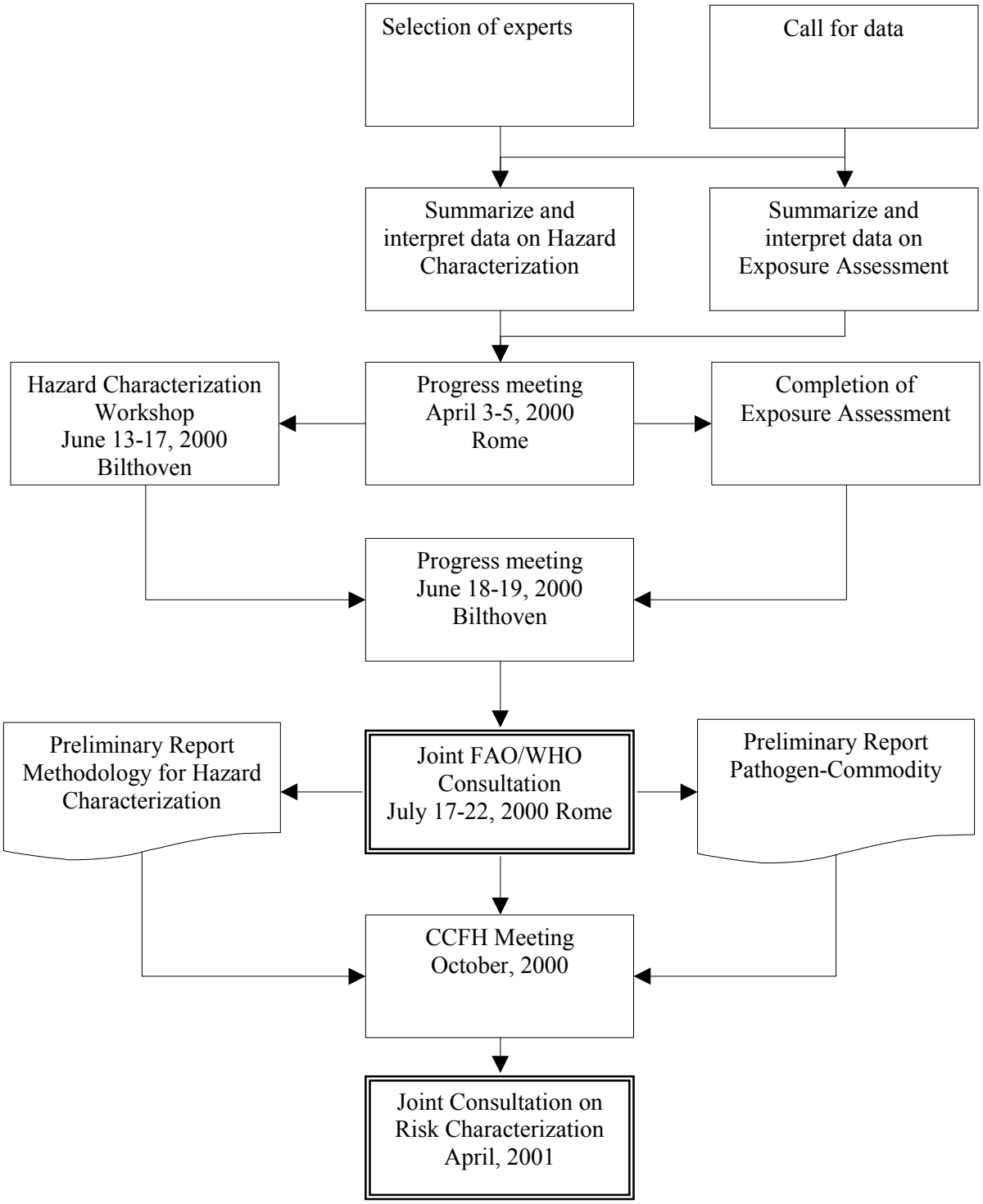
Annex 2: Joint FAO/WHO Microbiological Risk Assessment Activities

Process of Operation for the Joint FAO/WHO Activities on Risk Assessment of Microbiological Hazards in Foods.



Note: Activities relating to the development of guidelines for exposure assessment of microbiological hazards in foods are currently under consideration.

Annex 3: List of working documents

Six working papers were prepared for, and presented during the expert consultation. These served as the basis for the discussions, which led to the development of the report and the recommendations. These documents were prepared for FAO and WHO by a number of expert drafting groups. The full text of these documents is available on the FAO and WHO WebPages.
http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/pagerisk/riskpage.htm
http://www.who.int/fsf/mbriskassess/index.htm

Paper no.	Title	Authors
MRA 00/01	Hazard identification and hazard characterization of *Listeria monocytogenes* in ready-to-eat foods	Robert Buchanan, Food and Drug Administration, USA Roland Lindqvist, National Food Administration, Sweden
MRA 00/02	Exposure assessment of *Listeria monocytogenes* in ready-to-eat foods	Tom Ross, University of Tasmania, Australia Ewen Todd, Bureau of Microbial Hazards, Health Canada Mark Smith, Bureau of Biostatistics and Computer Applications, Health Canada
MRA 00/03	Hazard identification and hazard characterization of *Salmonella* in broilers and eggs	Aamir Fazil, Decisionalysis Risk Consultants, Canada Roberta A. Morales, Research Triangle Institute, USA Anna M. Lammerding, Microbial Food Safety Risk Assessment, Health Canada Andrea S. Vicari, North Carolina State University, USA Fumiko Kasuga, National Institute of Infectious Diseases Japan
MRA 00/04	Exposure assessment of *Salmonella* Enteritidis in eggs	Eric Ebel, United States Department of Agriculture Fumiko Kasuga, National Institute of Infectious Diseases Japan Wayne Schlosser, United States Department of Agriculture Shigeki Yamamoto, National Institute of Infectious Diseases Japan
MRA 00/05	Exposure assessment of *Salmonella* spp. in broilers	Louise Kelly, Veterinary Laboratories Agency, UK Wayne Anderson, Food Safety Authority of Ireland Emma Snary, Veterinary Laboratories Agency, UK
MRA 00/06	WHO/FAO Draft Guidelines on Hazard Characterization for Pathogens in food and Water	WHO/FAO/RIVM workshop on hazard characterization of pathogens in food and water, 13 – 17 June 2000, Bilthoven, The Netherlands