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Risk assessment of choleragenic *Vibrio cholerae* 01 and 0139 in warm-water shrimp in international trade

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FOREWORD

The Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food both at national and at international levels. Increasing foodborne disease incidence over the last decades seems, in many countries, to be related to an increase in disease caused by microorganisms in food. This concern has been voiced in meetings of the Governing Bodies of both Organizations and in the Codex Alimentarius Commission. It is not easy to decide whether the suggested increase is real or an artefact of changes in other areas, such as improved disease surveillance or better detection methods for microorganisms in foods. However, the important issue is whether new tools or revised and improved actions can contribute to our ability to lower the disease burden and provide safer food. Fortunately, new tools, which can facilitate actions, seem to be on their way.

Over the past decade, Risk Analysis – a process consisting of risk assessment, risk management and risk communication – has emerged as a structured model for improving our food control systems with the objectives of producing safer food, reducing the numbers of foodborne illnesses and facilitating domestic and international trade in food. Furthermore, we are moving towards a more holistic approach to food safety, where the entire food chain needs to be considered in efforts to produce safer food.

As with any model, tools are needed for the implementation of the risk analysis paradigm. Risk assessment is the science-based component of risk analysis. Science today provides us with in-depth information on life in the world in which we live. It has allowed us to accumulate a wealth of knowledge on microscopic organisms, their growth, survival and death, even their genetic make-up. It has given us an understanding of food production, processing and preservation, and of the link between the microscopic and the macroscopic worlds and how we can benefit from, as well as suffer from, these microorganisms. Risk assessment provides us with a framework for organizing all this data and information and to better understand the interaction between microorganisms, foods and human illness. It provides us with the ability to estimate the risk to human health from specific microorganisms in foods and gives us a tool with which we can compare and evaluate different scenarios, as well as identify the types of data necessary for estimating and optimizing mitigating interventions.

Microbiological risk assessment can be considered as a tool that can be used in the management of the risks posed by foodborne pathogens and in the elaboration of standards for food in international trade. However, undertaking a microbiological risk assessment (MRA), particularly quantitative MRA, is recognized as a resource-intensive task requiring a multidisciplinary approach. Yet foodborne illness is among the most widespread public health problems, creating social and economic burdens as well as human suffering, making it a concern that all countries need to address. As risk assessment can also be used to justify the introduction of more stringent standards for imported foods, a knowledge of MRA is important for trade purposes, and there is a need to provide countries with the tools for understanding and, if possible, undertaking MRA. This need, combined with that of the Codex Alimentarius for risk-

based scientific advice, led FAO and WHO to undertake a programme of activities on MRA at the international level.

The Food Quality and Standards Service, FAO, and the Food Safety Department, WHO, are the lead units responsible for this initiative. The two groups have worked together to develop the area of MRA at the international level for application at both the national and international levels. This work has been greatly facilitated by the contribution of people from around the world with expertise in microbiology, mathematical modelling, epidemiology and food technology, to name but a few disciplines.

This Microbiological Risk Assessment series provides a range of data and information to those who need to understand or undertake MRA. It comprises risk assessments of particular pathogen-commodity combinations, interpretative summaries of the risk assessments, guidelines for undertaking and using risk assessment, and reports addressing other pertinent aspects of MRA.

We hope that this series will provide a greater insight into MRA, how it is undertaken and how it can be used. We strongly believe that this is an area that should be developed in the international sphere, and have already from the present work clear indications that an international approach and early agreement in this area will strengthen the future potential for use of this tool in all parts of the world, as well as in international standard setting. We would welcome comments and feedback on any of the documents within this series so that we can endeavour to provide Member countries, Codex Alimentarius and other users of this material with the information they need to use risk-based tools, with the ultimate objective of ensuring that safe food is available for all consumers.

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BACKGROUND

In response to a request from Codex for scientific advice, FAO and WHO, in 2001, established a risk-assessment drafting group and convened an expert consultation to take the first steps in developing a risk assessment on *Vibrio* spp. in seafood products that would have the most impact on public health or international trade, or both. The expert consultation concluded that three species, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and choleragenic *Vibrio cholerae*, were the species responsible for most cases of human illness cased by vibrios, and several seafood vehicles associated with these illnesses were identified. Work was thus undertaken on the following pathogen-product combinations:

- *V. parahaemolyticus* in raw oysters harvested and consumed in Australia, Canada, Japan, New Zealand and the United States of America.
- *V. parahaemolyticus* in finfish consumed raw.
- *V. parahaemolyticus* in bloody clams harvested and consumed in Thailand.
- *V. vulnificus* in raw oysters harvested and consumed in the United States of America.
- Choleragenic *V. cholerae* O1 and O139 in warm-water shrimp in international trade.

These five individual risk assessments illustrate how different approaches were used to reflect the national capacity to generate data, including health statistics and data on the pathogen and the commodity of concern. The assessments considered information on *Vibrio* spp. in seafood that was generated and available at regional and national levels, and this information formed the substantive basis from which the risk assessments were developed.

The current document describes the risk assessment of choleragenic *Vibrio cholerae* O1 and O139 in warm-water shrimp in international trade. Choleragenic *V. cholerae* is an important pathogen in many developing countries, where it can cause devastating disease and economic burdens. Within developing cholera-endemic countries, the data needed for a quantitative risk assessment may not be available. However, there is growing information (data on food and human health) indicating that food from developing countries, especially shrimp, in international trade is not a risk for cholera. This risk assessment was undertaken to use the available data to address some of the problems faced by developing countries with respect to the export market for warm-water shrimp.

In undertaking the work, it was recognized that the risk of acquiring cholera from shrimp traded and consumed in the domestic market was not addressed. The lack of data made this impossible at the current time. However, the report provides different approaches to risk assessment in an effort to make this tool more easily adaptable for use at the national level and to estimate risk at the domestic level as and when appropriate data become available.

ABBREVIATIONS

%	percentage
APW	alkaline peptone water
CAC	Codex Alimentarius Commission
CCP	Critical Control Point
CFSAN	Center for Food Safety and Applied Nutrition, FDA
cfu	colony forming unit
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (United States of America)
g	gram
GHP	Good Hygienic Practice
GMP	Good Manufacturing Practice
h	hour(s)
HACCP	Hazard Analysis and Critical Control Point [system]
INFOFISH	Intergovernmental Organization for Marketing Information and Technical and Advisory Services for Fishery Products in the Asia-Pacific Region
ml	millilitre
$NaHCO_3$	Sodium bicarbonate (sodium hydrogen carbonate)
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
pH	Hydrogen ion concentration
ppt	parts per thousand
SSOP	Sanitation Standard Operating Procedures
t	tonne
TCP	toxin-co-regulated pilus
TCBS	Thiosulfate Citrate Bile-salt Sucrose
VBNC	Viable but non-culturable
WHO	World Health Organization

INTERPRETATIVE SUMMARY

INTRODUCTION

Outbreaks of cholera have been associated with consumption of seafood including oysters, crabs and shrimp (Oliver and Kaper, 1997). In the early 1990s, a pandemic of cholera swept through South and Central America. The outbreaks seemed to begin in Peru, where there were more than 400 000 cases and 4 000 deaths (Wolfe, 1992). However, the mortality rate may have been higher but for the readily available oral electrolyte stations throughout Latin America, established as a precaution when WHO anticipated the pandemic would jump from Africa to Latin America. Although no cases of cholera were associated with the consumption of commercial seafood, the industry, including shrimp exports, were negatively affected. The outbreak in the 1990s cost Peru US$ 770 million as a result of food trade embargos and adverse effects on tourism (WHO, no date). Similarly, the European Union (EU) banned importation of fish from eastern Africa as a result of an outbreak of cholera in the region. This ban lasted from late December 1997 until June 1998, even though opinions of the Food and Agriculture Organization of the United Nations and the World Health Organization rejected the restriction indicating it was "not the most appropriate response" (FAO, 1998).

Warm-water shrimp is an important commodity in international trade. In 1999, global production was about four million tonne, of which 1.3 million tonne were traded internationally, with three-quarters of this originating from developing countries (FAO, 1999), making it a very important commodity for these countries.

The Hazard Analysis and Critical Control Point (HACCP) system is a widely accepted food safety management system to assure the safety of food. The global shrimp trade has responded to the major HACCP initiatives of the United States of America (Seafood HACCP Regulation (FDA, 1995)) and of the European Union (concept of “own checks” and critical control points (EC, 1991)) as prerequisites for maintaining trade. In addition, while many importing countries operate microbiological monitoring systems at ports of entry, it is the responsibility of regulatory authorities in exporting countries to manage food safety risks in products at the individual company and process levels. It would therefore seem that the international food trade, as a whole, has become highly regulated in terms of food safety. The impact of these initiatives has been especially difficult for the international shrimp trade, with the perception of importing countries being that warm-water shrimp may be the source of foodborne pathogens, as highlighted by the example above, regarding Peru.

SCOPE

In light of the background described above, the scope of this work was to assess the risk of acquiring cholera as a result of consumption of imported warm-water shrimp. Although the potential health risk associated with the consumption of domestically produced and consumed shrimp was recognized, it was not considered in the current risk assessment. Domestically produced and consumed shrimp does not generally follow the same production-to-consumption chain as exported warm-water shrimp, and would need to be addressed separately. However, when undertaking this present assessment, there were inadequate data available to address the domestic production and consumption situation. Thus, the risk assessment focussed only on warm-water shrimp produced and processed for the export market. The risk assessment considered the risk associated with consumption of imported shrimp in several importing countries. Consumption of both uncooked shrimp (assumed to be 10% of total consumption),

probably as sashimi or sushi, and of shrimp cooked (assumed to be 90% of total consumption) either at the processing plant or as part of meal preparation, was taken into account.

RISK ASSESSMENT

Approach

A joint FAO/WHO drafting group was established to estimate the likelihood of contracting cholera from the consumption of warm-water shrimp in international trade containing choleragenic *Vibrio cholerae* O1 and O139. The group undertook a process of data gathering on the prevalence and concentration of choleragenic strains of *V. cholerae* at key points in the harvesting–processing–storage–preparation–consumption continuum. Much of the data available was qualitative in nature, and while a number of studies were available on the prevalence of choleragenic *V. cholerae* in water and shrimp, there was a deficit of data on the levels of the organism in shrimp or its environment. Although quantitative data were not available for many aspects of the harvest-to-consumption continuum, data from port-of-entry analysis of imported warm-water shrimp were available and used in this risk assessment. In addition, very little information was available on consumption. To overcome this, statistics on volumes of warm-water shrimp imported and population statistics for seven selected importing countries were used as a basis for calculating an estimate of the annual number of servings consumed.

Taking into consideration the available data, both qualitative and quantitative approaches to estimating the risk were considered and developed in the course of this work. The qualitative approach was elaborated based on an approach developed by Food Science Australia (FSA, 2000) to describe risk profiles of plant products. This qualitative approach took into consideration the harvest-to-consumption continuum. In addition, information published by the International Commission on Microbiological Specifications of Foods (ICMSF, 2002) on descriptors for severity of illnesses caused by various pathogens was used as a basis for describing the output of the hazard characterization.

Two different approaches were pursued in an effort to develop a more quantitative estimation of the risk. Both of these approaches focused specifically on seven countries that import shrimp, and thus some of the data inputs were specific to those countries. The first of these approaches was based on a published spreadsheet-based, food-safety risk-assessment tool (Ross and Sumner, 2002). This is a mathematical model with a user-friendly interface that undertakes calculations based on eleven specified inputs to develop indices of public health risk. It could, perhaps, be considered as a type of bridging risk assessment between a fully qualitative and fully quantitative approach. The second approach that was pursued involved the development a fully quantitative risk-assessment model specifically for this pathogen - commodity combination using the available quantitative data. As the numerical inputs for a full harvest-to-consumption model were not available, this model was based on a shortened exposure pathway that began at the port-of-entry in the importing country.

Hazard identification

According to the WHO definition, choleragenic *V. cholerae* O1 and O139 are the only causative agents of cholera, a water- and foodborne disease with epidemic and pandemic potential. Choleragenic *V. cholerae* causes mild to severe gastrointestinal illness, and may cause patient dehydration, leading to death. Common symptoms include profuse watery diarrhoea, anorexia

and abdominal discomfort. In cholera gravis, the rate of diarrhoea may quickly reach 500 to 1000 ml / hour, leading rapidly to tachycardia, hypotension and vascular collapse due to dehydration (Kaper, Morris and Levine, 1995). The primary source of choleragenic *V. cholerae* is the faeces of persons acutely infected with the organism and thus it reaches water most often through sewage. In aquatic environments a strong association between the levels of zooplankton and the incidence of *V. cholerae* has been observed (Huq et al., 1983) and this as well as other environmental factors may contribute to the seasonality of cholera (Colwell and Spira, 1992). *V. cholerae* can survive in water for long periods but appears to be confined to fresh water and estuarine environments. There are very few records of isolation of choleragenic *V. cholerae* from shrimp.

Exposure assessment

An overview of the harvest-to-consumption pathway to be considered was developed as presented in Figure 1. This assisted in the identification of the various points along the continuum that influence the prevalence and level of choleragenic *V. cholerae* in warm-water shrimp. Data on prevalence of choleragenic *V. cholerae* in water and shrimp indicated a range from 0 to 2%. However, studies rarely indicated the actual numbers of *V. cholerae* cells present. Port-of-entry testing data were available from three countries and these indicated two positive samples out of almost 22 000 warm-water shrimp samples tested, suggesting a prevalence of around 0.01% in exported warm-water shrimp. The impact of processing steps on choleragenic *V. cholerae* were quite substantial, resulting in reductions up to 6 logs depending on the step (washing, icing, cooking, freezing).

Very little information was available on consumption of imported warm-water shrimp. To overcome this, statistics on volumes of warm-water shrimp imported and population statistics for seven selected importing countries, i.e. France, Germany, Italy, Japan, Spain, United Kingdom and the United States of America, were used as a basis for calculating the annual number of servings consumed.

Hazard characterization

V. cholerae O1 and O139 can infect both adults and children causing diarrhoeal disease. About 20% of those who are infected develop acute, watery diarrhoea and 10 to 20 % of these individuals go on to develop severe watery diarrhoea with vomiting (WHO, 2004). Without prompt and adequate treatment severe dehydration and death can occur within hours and the case-fatality rate in untreated cases may reach 30-50%. However, treatment is straightforward and if applied appropriately the case-fatality rate is less that 1% (WHO, 2004).

Gastrointestinal illness, typical of cholera, was considered as the endpoint of this risk assessment. There are a number of reports indicating that the number of choleragenic organisms required to cause illness is in the region of one million cells and therefore this number was considered to be the threshold dose in developing the qualitative risk assessment approach, and also in using the published spreadsheet-based, risk-assessment tool. However, as human volunteer feeding trial data were also available for *V. cholerae*, these were used to develop a dose-response curve. Human volunteer data were available for both the Classical and El Tor biotypes. The dose-response curve was obtained by fitting the approximate Beta-Poisson model to data from the volunteer studies. However, as the El Tor biotype is now more commonly

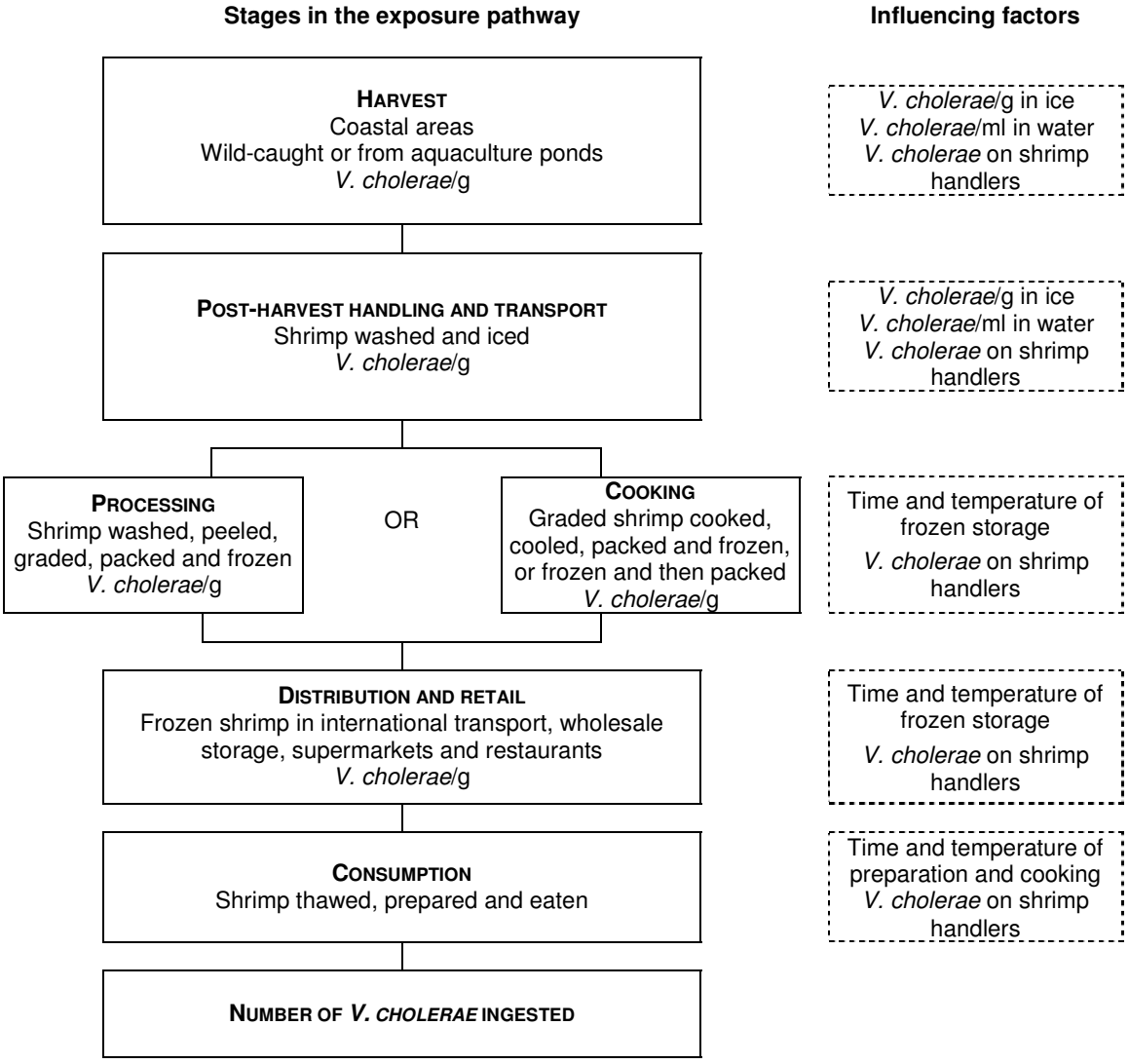

Figure 1. Production-to-consumption pathway for exposure assessment of *V. cholerae* in warm-water shrimp harvested and processed for international markets.

observed in relation to clinical cases of cholera, the dose-response model developed using data for this biotype was used in the risk assessment. This dose-response curve was subsequently used in the quantitative model developed in the course of this work.

Risk characterization

Estimations of the likelihood of acquiring cholera from the consumption of imported warm-water shrimp was generated using both qualitative and quantitative approaches.

In the qualitative risk assessment, a matrix embracing responses to a range of qualitative criteria was developed. This matrix considered the occurrence of illness associated with warm-water shrimp, severity of illness, whether growth of the hazard in the product was required to cause illness and the impact of various steps during processing and consumer preparation on the hazard. This analysis indicated that the opportunity was small for choleragenic *V. cholerae* to survive processing and therefore be present in shrimp that is finally consumed. The very low occurrence of illness and the lack of a documented epidemiological link support this. Categorization of this situation in terms of risk was not considered appropriate, as any descriptor used might be interpreted in different ways by different people. However, the presentation of information on the various relevant aspects provides an adequate basis for decision-making in some situations.

The published spreadsheet-based tool was used to estimate the likelihood of contracting cholera from consuming raw, warm-water shrimp in seven importing countries for which data were available. Based on the data used as inputs to the spreadsheet-based tool, 1 to 2 cases of cholera caused by consumption of warm-water shrimp in a decade was predicted for Japan, the United States of America, and Spain and approximately 1 case every 25 years in the other countries considered. This tool provides a user-friendly interface to facilitate the application of risk assessment in decision-making but it does have limitations, and the manner in which the estimates are calculated may not be easily understood. Nevertheless it has been found to be useful to risk managers, particularly in risk ranking exercises.

The fully quantitative risk assessment that was also developed focused on the pathway from the port-of-entry to the point of consumption. This quantitative model estimated that the median risk of acquiring cholera from warm-water shrimp in the selected importing countries ranged from 0.009 to 0.9 cases per year depending on the country. In addition, the risk assessment indicated that the median risk was between 2 and 9 illnesses from every thousand million (10^9) servings of warm-water shrimp. While the advantage of the quantitative approach is a numerical estimate of the risk, such risk assessments are only as good as the data that was used in their development. More detailed information on each of these approaches and the outcomes can be found in the technical report.

GAPS IN THE DATA

In undertaking this assessment, a number of gaps in our knowledge and database were identified. These included a lack of quantitative data on the levels of the microorganism at various points along the production-to-consumption chain. This gap is even greater if the domestic production-to-consumption chain is being considered. While extensive port-of-entry testing data were available for three countries, there was a lack of testing data for many of the importing countries considered in this risk assessment. Actual data on the levels of faecal cross-contamination during handling of shrimp, as well as post-processing or cross-contamination data, were not available. Consumption data for shrimp were not available and had to be approximated using volumes of shrimp imported into selected countries.

KEY FINDINGS

- *V. cholerae* is widely distributed in the environment. However, it is important to note that only those strains producing cholera toxin and belonging to serotypes O1 and O139 are causative agents of cholera. Such strains are rarely isolated in aquatic environments.
- The adherence of processors of shrimp for the export market to GMP/GHP/HACCP requirements minimises the potential for contamination and /or cross-contamination with, and subsequent multiplication of, choleragenic *V. cholerae* on either wild-caught or cultured shrimp during handling and processing.
- Significant log-reductions in numbers of choleragenic organisms occur during washing, freezing and cooking. Therefore, very low levels of *V. cholerae* might be found in imported shrimp.
- Extensive testing data from importing countries show that choleragenic *V. cholerae* O1 and O139 are rarely isolated in imported warm-water shrimp (2 in ~22 000 samples).
- Using human feeding trial data it was possible to develop dose-response curves for both the Classical and El Tor *V. cholerae* biotypes. These curves indicated a difference in the dose-response for each of the biotypes. This may be an artefact of the manner in which the data were collected or may indicate a difference in virulence of the two biotypes. As the El Tor is currently the most commonly occurring biotype the dose-response curve developed for this biotype was used in the estimation of risk in the quantitative risk assessment. For the qualitative risk assessment and the spreadsheet tool a dose of 10^6 *V. cholerae* cells was assumed to cause illness when consumed in food.
- While the qualitative risk assessment describes a situation where there appears to be little opportunity to acquire cholera from warm-water shrimp, the quantitative approaches support this by predicting low levels of risk of illness for some of the major consuming countries of imported warm-water shrimp. These results reflect the lack of documented cases of cholera attributable to internationally traded warm-water shrimp.

CONCLUSIONS

Only choleragenic *V. cholerae* O1 and O139 are the causative agents of cholera. While, non-O1, non-O139 *V. cholerae* may be occasionally found in shrimp, there is no known risk of cholera associated with these serotypes in shrimp or any other product. The risk assessments presented here estimated a very small risk of acquiring cholera through consumption of imported warm-water shrimp. This reflects the picture given by the available epidemiological data. However, further research to address the data gaps noted above needs to be conducted. Should such data become available, it would be relatively straightforward to modify the inputs to the approaches presented here to reflect new data. In the course of this work, the risk associated with domestically produced and consumed shrimp was not considered. While the qualitative approach and the spreadsheet-based tool presented here could be used as a basis to begin risk assessment in this area, the fully quantitative risk-assessment model developed is not appropriate for application to the domestic scenario.

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TECHNICAL REPORT

1. Introduction

Warm-water shrimp[1] is an important commodity in international trade (Table 1). The total world shrimp production in 1999 was about four million tonne, of which 1.3 million tonne were traded internationally, with three-quarters of this originating from developing countries (FAO, 1999). While cases of cholera in shrimp-producing countries often have a negative effect on shrimp exports, to date there have been no documented cases of cholera caused by internationally traded warm-water shrimp. FAO has comprehensive data on the quantity, origin and destination of shrimp traded between different countries. Regulatory agencies in some of the top shrimp importing countries, such as the United States of America and Japan, routinely test imported warm-water shrimp samples for the presence of choleragenic *Vibrio cholerae*, and test data for the period 1995–2000 were available for estimation of the prevalence and distribution of this pathogen in warm-water shrimp. These data have been used for the risk assessment work presented in this report.

It must be emphasized at the outset that the present risk assessment work is concerned solely with warm-water shrimp in international trade. It is acknowledged that cholera is a significant public health concern for many developing countries; however, there are major difficulties and uncertainties in defining handling and storage practices, possible routes of faecal cross-contamination and consumption practices for domestic shrimp. Also, only limited test data of the occurrence of choleragenic *V. cholerae* O1 and O139 in domestically consumed shrimp are available at market and retail levels.

Table 1. The top ten shrimp importing countries and their total volume of imports and cases of cholera in 2000.

Country	Shrimp imports		Total cholera cases in 2000 (imported cases)
	tonne $\times 10^3$	US$ million	
USA	345.7	3 848.7	4 (4)
Japan	283.0	3 167.0	34 (32)
Spain	114.7	767.6	1 (1)
Denmark	94.8	332.6	–
United Kingdom	77.9	540.0	33 (33)
France	67.7	495.2	–
Canada	66.4	377.8	–
Italy	49.6	344.8	–
The Netherlands	40.3	258.6	–
Hong Kong (SARC)	40.1	230.9	9 (3)

NOTES: Import figures include warm- and cold-water shrimp of all product types, i.e. fresh, chilled, frozen, canned, etc.

SOURCES: Import figures from FAO-Globefish, 2002. Cholera cases from WHO, 2001.

[1] The term shrimp is used for both wild-caught and cultured shrimp, and also includes product referred to as prawn.

1.1. Management approaches in the export of warm-water shrimp

Over the past two decades, the Hazard Analysis and Critical Control Point (HACCP) system has become essentially a prerequisite for companies and countries wishing to participate in international trade. *"HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing."* (CAC, 1997a, b). HACCP can be applied to the entire food chain from production-to-consumption. It is applied by companies to produce safe food, and by regulators as a way to aid inspection and to protect public health. Currently, HACCP combined with good hygiene practices (GHPs), good manufacturing practices (GMPs) and Sanitation Standard Operating Procedures (SSOPs) constitute an integrated food safety management system, as practised by food (seafood) companies involved in international trade (e.g. FDA, 2001a).

The global shrimp trade has responded to the major HACCP initiatives of the United States of America (Seafood HACCP Regulation (FDA, 1995)) and of the European Union (concept of "own checks" and critical control points (EC, 1991)) as prerequisites for maintaining trade. Additionally, the major importing trading blocs and countries operate microbiological monitoring systems at ports of entry, where a positive finding results in the exporting company being placed on a more stringent testing programme, which not only increases costs of production but also increases the likelihood of further detection. Major importing countries also place responsibility on regulatory authorities in exporting countries to manage food safety risks in products at the individual company and process levels. For example, the European Union (EU) requires each exporting country to have a competent authority to regulate food safety in accordance with EU Directives.

From the foregoing, it can be seen that the international food trade, as a whole, has become highly regulated in terms of food safety. The impact of these initiatives has been especially difficult for the international shrimp trade because of a long-held perception of importing countries that warm-water shrimp may be a source of foodborne pathogens. For example, in the late 1970s, the United States of America imposed a system of "blacklisting" on shrimp exports from some Asian countries, placing their products on intensive sampling and monitoring regimes. In 1980, Asian cooked shrimp were implicated in an outbreak of shigellosis in the Netherlands (Bijkerk, 1984) though it was never established whether the product was contaminated in Asia during processing, or in the Netherlands during final preparation. The EU imposed blacklisting on shrimp exports from Bangladesh, because of a perception that *V. cholerae* was likely to be transferred to the product by food handlers. A cholera epidemic swept through Latin America in the early 1990s (Tauxe et al., 1994a), and although no cases of cholera were associated with the consumption of commercial seafood, the outbreak cost Peru US$ 770 million due to food trade embargos and adverse effects on tourism (WHO, 2003). Similarly, the EU banned importation of fish from eastern Africa as a result of an outbreak of cholera in the region. This ban lasted from late December 1997 until June 1998, even though expert opinion from FAO and WHO rejected the restriction based on human health concerns (FAO, 1998).

It is against this background that the international shrimp trade in general, and the warm-water shrimp trade in particular, developed HACCP systems to maintain food safety requirements. Irrespective of whether the primary source is marine or aquaculture product, the

process has developed a number of Critical Control Points (CCPs) for target pathogens, particularly those of faecal origin (e.g. *Salmonella, Shigella, V. cholerae*). These include:

- Primary chilling immediately after harvest in an ice-water slurry on vessels and at harvest sites.
- In cooked products, applying time-temperature regimes to give log reductions far in excess of likely contamination levels at sites of microbiological concern.
- Rapid chilling after cooking.
- Plate freezing, followed by frozen storage.

In addition, GHPs and GMPs for plant construction, water supply, ice production, temperature control and product flow from "dirty" to "clean" areas were adapted to conform with Codex requirements, and SSOPs exist for cleaning of food contact surfaces and for personal hygiene of handlers (CAC, 1997a, b).

The international shrimp trade sector, thus, is highly regulated by controlling authorities and heavily monitored by importing countries. The results of these monitoring programmes form the basis for establishing exposure to *V. cholerae* in the present risk assessment work.

2. Statement of purpose

The purpose of the present risk assessment work was to estimate the likelihood of consumers in selected countries contracting cholera following consumption of imported warm-water shrimp. The work focused on shrimp that had been harvested and processed specifically for international trade, and considered the likelihood of acquiring cholera in a country that imported such shrimp.

The work presented in this document follows the principles and guidelines for the conduct of microbiological risk assessment outlined by Codex (CAC, 1999). Hazard identification, exposure assessment, hazard characterization and risk characterization steps were undertaken. The information available for this risk assessment is primarily described in the sections on the exposure assessment and hazard characterization. Due to the nature of the available data, the exposure assessment from harvest to the point of consumption is primarily descriptive. A second, shorter, exposure pathway, beginning at the point of import, and for which some numerical data were available, is also described. The hazard characterization step also included the development of a dose-response model. Therefore, these two steps include both textual and numerical outputs.

The risk characterization step differs somewhat from risk assessments previously described in this series as it describes three approaches, ranging from qualitative to quantitative, for combining the information from the two previous steps to provide a description of the risk. The assumptions used therein and the advantages and limitations associated with each of these approaches are also described.

In order to facilitate the estimation of consumption, seven importing countries for which relevant data were available were selected for consideration in this risk assessment. These were France, Germany, Italy, Japan, Spain, United Kingdom and the United States of America. They were selected to provide an example of how such risk assessment work could be undertaken and how the approaches used could be applied to other countries importing warm-water shrimp through substitution of the data used with data specific to the particular country of concern.

This work does not address the risk associated with the domestic consumption of warm-water shrimp. It is recognized that warm-water shrimp for the domestic market is usually harvested and processed under very different conditions. However, the lack of data in that area meant that it was not possible at this stage to undertake a risk assessment to estimate the likelihood of consumers contracting cholera following consumption of domestically harvested and prepared warm-water shrimp. Nevertheless, should such data become available the qualitative approach and the spreadsheet-based tool presented here could be used as a basis to begin risk assessment in this area; the fully quantitative risk-assessment model developed herein is not appropriate for application to the domestic scenario.

3. Hazard identification

3.1. Presence of the organism

3.1.1. *V. cholerae* serovars of concern

According to the WHO definition, choleragenic *V. cholerae* O1 and O139 are the only causative agents of cholera, a water- and foodborne disease with epidemic and pandemic potential. Other serogroups (serovars) of *V. cholerae* are generally termed non-O1, non-O139 strains. They are generally non-choleragenic, usually cause a milder form of gastroenteritis than O1 and O139, and are normally associated with sporadic cases and small outbreaks rather than with epidemics and pandemics (Kaper, Morris and Levine, 1995; Borroto, 1997; Desmarchelier, 1997). The O1 serovar is classified into three antigenic forms: Inaba, Ogawa and Hikojima. These antigenic forms are referred to as subtypes of *V. cholerae* O1 strains and can be classified into two biotypes, Classical and El Tor, based on their phenotypic characteristics (Kaper, Morris and Levine, 1995). Recent studies have shown that the Classical biotype strains are rarely isolated from any part of the world (Sack et al., 2003). The choleragenic El Tor biotype strains of *V. cholerae* are grouped in four major clonal groups: (i) the seventh pandemic; (ii) the U.S. Gulf Coast; (iii) Australia; and (iv) Latin America (difficult to distinguish from the seventh pandemic strain and produces a very similar PFGE pattern), which seem to reflect broad demographic and epidemiological associations (Wachsmuth et al., 1994).

This risk assessment focuses primarily on *V. cholerae* O1, since very limited information was available for *V. cholerae* O139. Further, there is little evidence that the two serovars (O1/O139) differ in relation to exposure assessment and hazard characteristics. Also, cholera outbreaks associated with the O139 serovar have not been reported outside South-east Asian countries, and the importance of the O139 serotype as a cause of cholera, even in these countries, has decreased in recent years (Ramamurthy et al., 2003).

The most important virulence factor associated with *V. cholerae* O1 and O139 is the cholera toxin. The *ctx* genes (*ctx*A and *ctx*B) encoding the production of the cholera toxin have been sequenced and this has enabled development of DNA probes and polymerase chain reaction (PCR) methods for detection of this gene in isolates of *V. cholerae* (Shirai et al., 1991; Koch et al., 1993; Olsvik et al., 1993; Karunasagar et al., 1995). This has enabled specific detection of choleragenic *V. cholerae* from seafood and water. Non-choleragenic *V. cholerae* O1 has been isolated from the environment in several studies (Colwell, Kaper and Joseph, 1977; Kaper et al., 1979; Colwell et al., 1981; Sakazaki and Donovan, 1984; Martins et al., 1991; Minami et al., 1991; Dalsgaard et al., 1995b). Serotyping of *V. cholerae* isolates from seafood does not provide adequate information for risk assessment. Shimada, Sakazaki and Oue (1987) reported that some non-pathogenic environmental vibrios may cross-react with polyvalent O1 antiserum, leading to misidentification. Dalsgaard, Mazur and Dalsgaard (2002) noted that *V. cholerae* O155 isolated from warm-water shrimp cross-reacts with O139 antiserum, leading to misidentification with the O155 serotype isolates lacking virulence-associated genes. Therefore, were *V. cholerae* O1 and O139 strains to be isolated from shrimp or other commercial products, it should be determined whether they contain *ctx* genes.

In addition to cholera toxin, choleragenic strains of *V. cholerae* possess the ability to adhere to, and colonize, the small intestine (colonization factor), which has been attributed, *inter alia*, to a toxin-co-regulated pilus (TCP). Genes encoding major virulence-associated factors are found in clusters (Hacker et al., 1997). It has been shown that *ctx* genes form part of a filamentous bacteriophage designated CTX phage (Waldor and Mekalanos, 1996; Faruque, Albert and Mekalanos, 1998). The pilus colonization factor is also known to act as a receptor for the CTX phage (Waldor and Mekalanos, 1996) and is encoded by the *tcp*A gene that is part of the *V. cholerae* pathogenicity island (Karaolis et al., 1998). The emergence of the O139 serotype as a choleragenic strain has provided a unique opportunity to study the evolution of new epidemic strains. Molecular epidemiological studies suggest that O139 strains are closely related to O1 El Tor strains. Conversion of the ancestral El Tor strain involved insertion of a large foreign genomic region encoding the O139 antigen-specific genes and simultaneous deletion of most of the O1 antigen-specific genes (Faruque et al., 2003). Several investigators have studied the presence of virulence-associated genes in environmental strains of non-O1, non-O139 *V. cholerae* (Chakraborty et al., 2000; Rivera et al., 2001). Though *ctx*-positive non-O1, non-O139 strains have been found, these strains often lack the full set of virulence genes found in epidemic strains. Chakraborty et al. (2000) noted absence of *tcp*A genes in *ctx*-positive strains, while Rivera et al. (2001) noted absence of genes encoding zonula occludens toxin (*zot*). A multiplex PCR amplifying *tcp* and *ctx* genes has been suggested for detecting choleragenic *V. cholerae* O1/O139 from aquatic ecosystems in cholera surveillance programmes (Rivera et al., 2003). Although *ctx*-positive non-O1, non-O139 serovars of *V. cholerae* have been implicated in cholera-like disease, only sporadic cases have been reported (Dalsgaard et al., 2001). Thus, the present evidence does not indicate that the emergence of new choleragenic serovars is of serious concern in the safety of shrimp product.

3.1.2. Prevalence of *V. cholerae* O1 and O139 in shrimp and water

Cholera is exclusively a human disease and no animal species has been found consistently infected. The primary source of *V. cholerae* O1 and O139 is faeces of persons acutely infected with the organism. The organism reaches water most often through sewage. The presence of the organism in the aquatic environment is not directly correlated with the presence of faecal coliform bacteria, but nutrients discharged with human sewage may enhance the survival of *V. cholerae*. The organism can survive in water for long periods and there are numerous instances where water has been implicated by epidemiological studies as a vehicle of *V. cholerae* O1. Examples of some of these are presented in Table 2.

The survival time of *V. cholerae* in water has been estimated (Table 3). The average time for a 1-log decline in cell number (t_{90}) is a function of the organism as well as the biotype (Feachem, Miller and Drasar, 1981) as shown in Table 3. Further work by the same researchers indicate that *V. cholerae* O1 is able to survive for extended periods in warm-water containing no nutrients but having a salinity of 0.25–3.0% and a pH of around 8 (Miller, Drasar and Feacham, 1984). The work of Colwell and Spira (1992) has shown that *V. cholerae* O1 can survive in water almost indefinitely and can be said to be an autochthonous aquatic organism. The conclusion that *V. cholerae* O1 can persist for long periods of time in water is supported by the observation that *V. cholerae* O1 of the same biotype, serotype, phage type and toxin profile has been isolated over a 30-year period in locations such as the Gulf of Mexico (Blake et al., 1983; Shandera et al., 1983). In Australia, *V. cholerae* O1 could be isolated intermittently over a 22-

month period from river water that was used as an auxiliary town water supply and implicated in a case of cholera in 1977 (Rogers, Cuffe and Cossins, 1977). However, *V. cholerae* O1 and O139 are confined to fresh water and estuarine environments, and there are no reports of the presence of these organisms in offshore environments.

In the aquatic environment, strong association between levels of zooplankton and incidence of *V. cholerae* has been observed (Huq et al., 1983). Adhesion to chitin has been shown to influence strongly the ecology of *V. cholerae* (Nalin et al., 1979). The organism is chitinolytic and its ability to digest chitin seems to play a role in its persistence in the environment (Dastidar and Narayanaswami, 1968; Colwell and Spira, 1992; Araújo et al., 1996). Choleragenic *V. cholerae* has also been reported to attach to the hindgut of crabs (Huq et al., 1996a) and it is noted that the hindgut of crustaceans is an extension of the exoskeleton and is lined with chitin.

Based on studies in Bangladesh, Colwell and Spira (1992) concluded that seasonality of cholera may be explained in that primary transmission is controlled by environmental factors such as temperature, salinity, nutrient concentration and zooplankton blooms, as well as by seasonal variation in seafood harvesting and consumption and by direct water contact. Studies in Bangladesh show that simple filtration of drinking water through sari cloth that removed zooplankton, most phytoplankton and particulates with a size >20μm was effective in removing 99% of *V. cholerae* (Huq et al., 1996b). Deployment of this filtration procedure in 65 villages in

Table 2. Some examples where water has been implicated by epidemiological studies as a vehicle of choleragenic *V. cholerae.*

Year	Country of isolation	Implicated water	Reference
1974	Portugal	Commercially bottled non-carbonated spring water, well water	Blake et al., 1977a, 1977b
1980	Thailand	Ice	Morris et al., 1982
1981	South Africa	River water	Sinclair et al., 1982
1984	Mali	Well water	Tauxe et al., 1988
1990	Malawi	Water stored at home, well water	Swerdlow et al., 1991b
1991	Bolivia	River water	Gonzales et al., 1992
1991	Ecuador	Street vendor drinks	Weber et al., 1994
1991	Peru	Street vendor drinks	Swerdlow et al., 1992
1991	Peru	Municipal water stored at home	Ries et al., 1992
1991	Peru	Ice	Ries et al., 1992
1992	Peru	Municipal water	Swerdlow et al., 1992
1998	Brazil	River water	Colaco et al., 1998
2000	Marshall Islands	Stored drinking water	Beatty et al., 2004
2002	India	Drinking water supply	Taneja et al., 2003

Table 3. Survival of *V. cholerae* in water

Biotype	Water	Mean (range) T_{90} (h)
Classical	Fresh water (non-sterile)	18 (0.16–36)
	Seawater (non-sterile)	95 (0.36–161)
El Tor	Fresh water (non-sterile)	53 (1–230)
	Seawater (non-sterile)	56 (8–235)

NOTE: T_{90} = the time in hours required for a 1-log reduction in *V. cholerae.*
SOURCE: Feachem, Miller and Drasar, 1981.

Bangladesh with a population of about 133 000 individuals yielded a 48% reduction in cases of cholera (Colwell et al., 2003). From the foregoing, it can be concluded that choleragenic *V. cholerae* is mainly found associated with plankton in the upper part of the water column or in the sediment where particulates with which the choleragenic *V. cholerae* is associated have settled.

There are very few records of isolation of *V. cholerae* O1 and O139 from shrimp. Studies from South-east Asia indicate absence of *V. cholerae* O1 from raw shrimp (Karunasagar et al., 1990, 1992; Fonseka, 1990; Rattagool et al., 1990). Several studies on shrimp farms in India indicated an absence of choleragenic *V. cholerae* in shrimp culture ponds (Nayyar Ahmad, Karunasagar and Karunasagar, 1995; Bhaskar et al., 1998; Otta, Karunasagar and Karunasagar, 1999; Shetty, 1999; Darshan, 2000; Gopal et al., 2005). Dalsgaard et al. (1995a) found that *V. cholerae* O1 was present in 2% (2/107) of water, sediment and shrimp samples collected from a major shrimp culture area in South-east Asia. However, subsequent testing of the isolates indicated absence of the *ctx* genes in both of the O1 strains (Dalsgaard et al., 1995b). During the 1997–1999 cholera epidemics in Sarawak, Malaysia, a study found that among 97 seafood isolates of *V. cholerae* O1 tested for *ctx,* 20 strains contained the gene and produced cholera toxin, of which 14, 1 and 5 of these toxigenic strains belonged to the O139, O1 Ogawa, and rough serotypes, respectively (Chen et al., 2004; Elhadi et al., 2004). The 20 toxigenic strains were isolated from various kinds of seafood collected at various locations in Malaysia. The isolation techniques used in this study included enrichment at 42°C, as well as PCR techniques, to screen for positive samples. Such methodology may lead to a higher level of detection and it is worth considering that the incidence of *V. cholerae* O1 and O139 in seafood, including shrimp, may be higher than reported due to limitations in the detection methodology. Data from India showed the presence of *V. cholerae* O1 in 0.2% of raw shrimp (Ministry of Agriculture, India, personal communication, 2001). However, the choleragenic status of these shrimp-associated strains is unknown. Shrimp imported into Europe in early 2005 tested positive for *V. cholerae*; but the subsequent detailed analysis indicated that they were non-toxigenic strains (see Appendix C). Data submitted to FAO/WHO from Argentina (M. Costagliola, personal communication, 2001) indicate the absence of *V. cholerae* O1 and O139 in 400 shrimp and 15 water samples examined.

In laboratory experiments, adhesion and colonization of *V. cholerae* O1 on shrimp and crab carapace was influenced by temperature and salinity (Castro-Rosas and Escartin, 2002). Both adhesion and colonization were optimal at salinity of 10–15 ppt, pH 6–7 and 37°C. At a salinity of 30 ppt, adhesion and colonization were considerably reduced, suggesting that conditions in wild shrimp seawater environments (pH 8.5 and salinity of 30 ppt) are not favourable for *V. cholerae* O1 to colonize the exoskeleton of shrimp in their natural habitat. In contrast, the salinity in aquaculture environments varies from 1 to 35 ppt and thus, in some instances, may provide a suitable environment for adhesion and colonization by *V. cholerae* O1.

In the aquatic environment, it has been suggested that *V. cholerae* enters a viable but non-culturable (VBNC) state, mainly due to low temperatures (Colwell and Spira, 1992). While recognizing that water and shrimp samples negative for *V. cholerae* may have this organism in the VBNC state, well-defined seasonal patterns of cholera epidemics negate the hypothesis that *V. cholerae* O1 and O139 not recovered by normal culture techniques are associated with the incidences of cholera.

Crustaceans, molluscs and finfish prepared in a variety of forms have been vectors for the transmission of *V. cholerae*. Table 4 cites some instances where seafood has been implicated in the transmission of *V. cholerae. V. cholerae* can be readily transmitted by consumption of raw seafood, especially molluscan shellfish (De Paola, 1981). There is one outbreak linked to the consumption of raw shrimp in the United States of America in 1986, where the source was domestic (Lowry et al., 1989). Another outbreak in Japan in 1978 was associated with lobsters imported from Indonesia (IASR, 1998). There was one other cholera outbreak linked to the consumption of raw shrimp, in the Philippines in 1962, though, since the source of shrimp is not known, it is not possible to assess whether *V. cholerae* O1 was naturally present or there as a result of cross-contamination after harvest (Joseph et al., 1965). The shellfish most often associated with cholera cases are molluscan shellfish (oysters) and crabs. While oysters are consumed raw in many countries, crabs are generally cooked, though even after boiling crabs for up to 10 minutes or steaming for up to 30 minutes, *V. cholerae* O1 may still retain viability (Blake et al., 1980).

Table 4. Selected examples of seafood implicated as vehicles for transmission of *V. cholerae*.

Site	Year	Product involved	Reference
Philippines	1962	Raw shrimp	Joseph et al., 1965
Malaysia	1971	Shellfish	Dutt, Alwi and Velauthan, 1971
Italy	1973	Raw mussels	Baine et al., 1974
Portugal	1974	Raw and undercooked shellfish	Blake et al., 1977b
Guam	1974	Salted raw fish	Merson et al., 1977
Gilbert Islands	1977	Raw and salted fish and clams	McIntyre et al., 1979
USA	1978	Cooked crabs	Blake et al., 1980
Japan	1978	Lobsters	IASR, 1998
Singapore	1982	Cooked squid	Goh et al., 1984
USA	1986	Cooked crab, cooked or raw shrimp	Lowry et al., 1989
Guinea-Bissau	1987	Cooked crab	Shaffer et al., 1988
USA	1998	Raw oyster	CDC, 1989
Chuuk (Truk)	1990	Raw fish	Swerdlow et al., 1991a
Ecuador	1991	Seafood	CDC, 1991
Japan	1991	Imported clams	Anon., 1991
USA	1992	Cooked crabs	Finelli et al., 1992
Hong Kong	1994	Seafood	Kam et al., 1995
Italy	1994	Raw fish and mussel	Maggi et al., 1994
USA (Colorado)	1998	Gulf coast Blue crab	Steinberg et al., 2001
Island of Pohnpei, Guam	2000	Reef fish	Haddock, Truong and Aguon, 2002
Berlin, Germany	2001	Fresh fish from Nigeria	Schurmann et al., 2002

3.2. Characteristics of the organism

3.2.1. Growth and survival characteristics

The physicochemical factors limiting the growth of *V. cholerae* O1 have been summarized by ICMSF (1996). The optimum temperature for growth is 37°C with a range of 10° to 43°C. The pH optimum for growth is 7.6 and *V. cholerae* can grow in a pH range of 5.0 to 9.6. The ability to grow under alkaline conditions is utilized in standard isolation procedures, when food samples are pre-enriched in alkaline peptone water (APW), which has a pH of 8.6. The water activity optimum for growth is 0.984 and growth can occur between 0.970 and 0.998. *V. cholerae* can grow in a salt range of 0.1–4.0% sodium chloride (NaCl), with an optimum for growth of 0.5% NaCl.

Kolvin and Roberts (1982) measured growth of *V. cholerae* O1 in raw and cooked seafood. No growth was observed in raw prawns, mussels and oysters, but growth occurred in cooked shellfish. Levels of 10^{10} cells/g were reported in cooked prawns and mussels stored at 37°C. At 22°C, there was a lag phase of 8 hours for the Classical biotype and 4 hours for the El Tor biotype. However, the results of this study on growth of *V. cholerae* on cooked prawns and mussels done by Kolvin and Roberts (1982) should be confirmed because the reported densities of 10^{10} cells/g shrimp are difficult to obtain in laboratory broth cultures, even under optimal growth conditions.

3.2.2. Death or inactivation

V. cholerae O1 is highly sensitive to acidic environments and is killed within minutes in gastric juice with pH <2.4. Therefore, normochlorohydric individuals are less susceptible to cholera, provided the food matrix does not protect the organisms. *V. cholerae* O1 is also highly sensitive to desiccation, indicating the need to use well-dried containers in product handling to minimize the transmission of cholera. This organism is heat sensitive, with a D-value of 2.65 minutes at 60°C (ICMSF, 1996).

The literature on survival of *V. cholerae* O1 in foods indicates different patterns of decline and longevity during storage at refrigeration and freezing temperatures (Felsenfeld, 1974). Careful interpretation of results, as also recommended by ICMSF (1996), is required to account for methodological differences, including age of inoculum, preparation of food substratum, application of inoculum, enumeration procedure and medium.

Most studies indicate that, while decline occurs at refrigeration temperatures, a proportion of the bacterial population remains viable. Pesigan, Plantella and Rolda (1967), starting with 10^5 cfu/g *V. cholerae* O1 in raw shrimp, recorded viable cells after 4–9 days at 5–10°C. Reilly and Hackney (1985) reported survival after 21 days at 7°C from an initial density of 7.8 log cfu/g. *V. cholerae* O1 inoculated at 10^3–10^4 cfu/g in ceviche, a marinated, ground or diced fish product, and stored at 8°C or 20°C, remained viable beyond the shelf life of the product at both temperatures (Torres-Vitela et al., 2000).

With respect to frozen storage, ICMSF (1996) reviewed literature from the 1930s that reported persistence for about 180 days and suggested that survival on fish was longer than on ground beef or vegetables. Nascumento et al. (1998), in contrast, reported a 6-log reduction on shrimp in 30 days at -20°C. In this study, samples were inoculated by immersion in a *V. cholerae* O1 suspension for 5 minutes, followed immediately by freezing to -20°C. Survivors were enumerated by direct plating on Thiosulfate Citrate Bile-salt Sucrose (TCBS) with incubation at 35°C. Both the method of inoculation, with organisms located on a water film on the surface of shrimp, and recovery on a highly selective medium, could contribute to the observed rapid decline. A qualitative study, at temperatures above and below freezing, in which survivors were recovered by enrichment before plating on TCBS agar and colonies confirmed by biochemical and serological testing, was reported by Corrales, Bainotti and Simonetta (1994). In fresh foods, including freshwater fish, *V. cholerae* O1 remained viable up to 90 days at -5°C and 30 days at -25°C. At non-freezing temperatures, survival time in fresh foods (milk, beef, fish and chicken) decreased with increasing temperatures: 18–20 days at 7°C; <10 days at room temperature; <2 days at 35°C (Corrales, Bainotti and Simonetta, 1994). As the food samples contained other bacteria, they spoiled rapidly at elevated temperatures and spoilage

organisms would have developed rapidly to the maximum population density supported by the product.

4. Exposure assessment

4.1. Introduction

Exposure assessment involves estimation of the likelihood of ingesting choleragenic *V. cholerae* O1 and O139 by eating shrimp contaminated with these organisms, and the numbers of the organisms consumed. Since most of the world's shrimp production and processing occurs in developing countries in Asia and Latin America, where cholera may be endemic, there can be multiple modes of contamination. Therefore, exposure assessment should consider possibilities of contamination and changes in population during pre-harvest, harvest, post-harvest handling and processing, retail, and at household level during preparation for consumption. Information and data for such a production-to-consumption chain approach is described below. An illustration of this is shown in the model for exposure to choleragenic *V. cholerae* O1 and O139 (Figure 1).

4.2. Overview of the production-to-consumption chain

4.2.1. Occurrence of choleragenic *V. cholerae* O1 and O139

Wild-caught shrimp

There is no evidence to show that marine shrimp caught by trawling in offshore waters with salinities of about 30 ppt harbour choleragenic *V. cholerae* O1 and O139. Choleragenic *V. cholerae* occur in waters with salinities between 0.2 and 20 ppt (Colwell and Spira, 1992). Studies conducted on freshly harvested marine shrimp indicate absence of choleragenic *V. cholerae* (Suseela et al., 1988; Iyer, Varma and Gopakumar, 1988; Fonseka, 1990; Karunasagar et al., 1990, 1992; Rattagool et al., 1990; Dalsgaard et al., 1995b).

Aquaculture shrimp

The contribution of aquaculture to shrimp production is increasing in most countries, and currently accounts for about a quarter of all shrimp production (FAO-Globefish, 2003). Most shrimp aquaculture activities are in coastal areas and the water source is generally estuaries or bays. In this situation, introduction of *V. cholerae* O1 or O139 is possible in cholera-endemic areas. However, studies conducted in several Asian countries indicate absence of choleragenic *V. cholerae* in shrimp from aquaculture ponds (Reilly and Twiddy, 1992; Nayyar Ahmad, Karunasagar and Karunasagar, 1995; Bhaskar et al., 1998; Otta, Karunasagar and Karunasagar, 1999; Shetty, 1999; Darshan, 2000; Dalsgaard et al., 1995b; Gopal et al., 2005). While no quantitative data could be found on densities of *V. cholerae* O1 and O139 in aquaculture environments or in shrimp, failure to isolate it in 25-g samples suggest a relatively low prevalence and density. Certainly, the densities (46 cfu / litre) of these organisms reported in natural waters (Colwell and Spira, 1992) also suggest that their densities in cultured shrimp will be relatively low. *V. cholerae* O1 has been shown to adhere to chitin and association has been demonstrated with zooplankton and crabs. The observation that shrimp is rarely associated with outbreaks of cholera suggest that the shrimp body surface and gut are not a preferred habitat for *V. cholerae* in natural waters. Ravi Kiran (1992) and Dalsgaard et al., (1995a) analysed shrimp

gut content for the presence of potential human pathogens and noted the absence of *V. cholerae* O1.

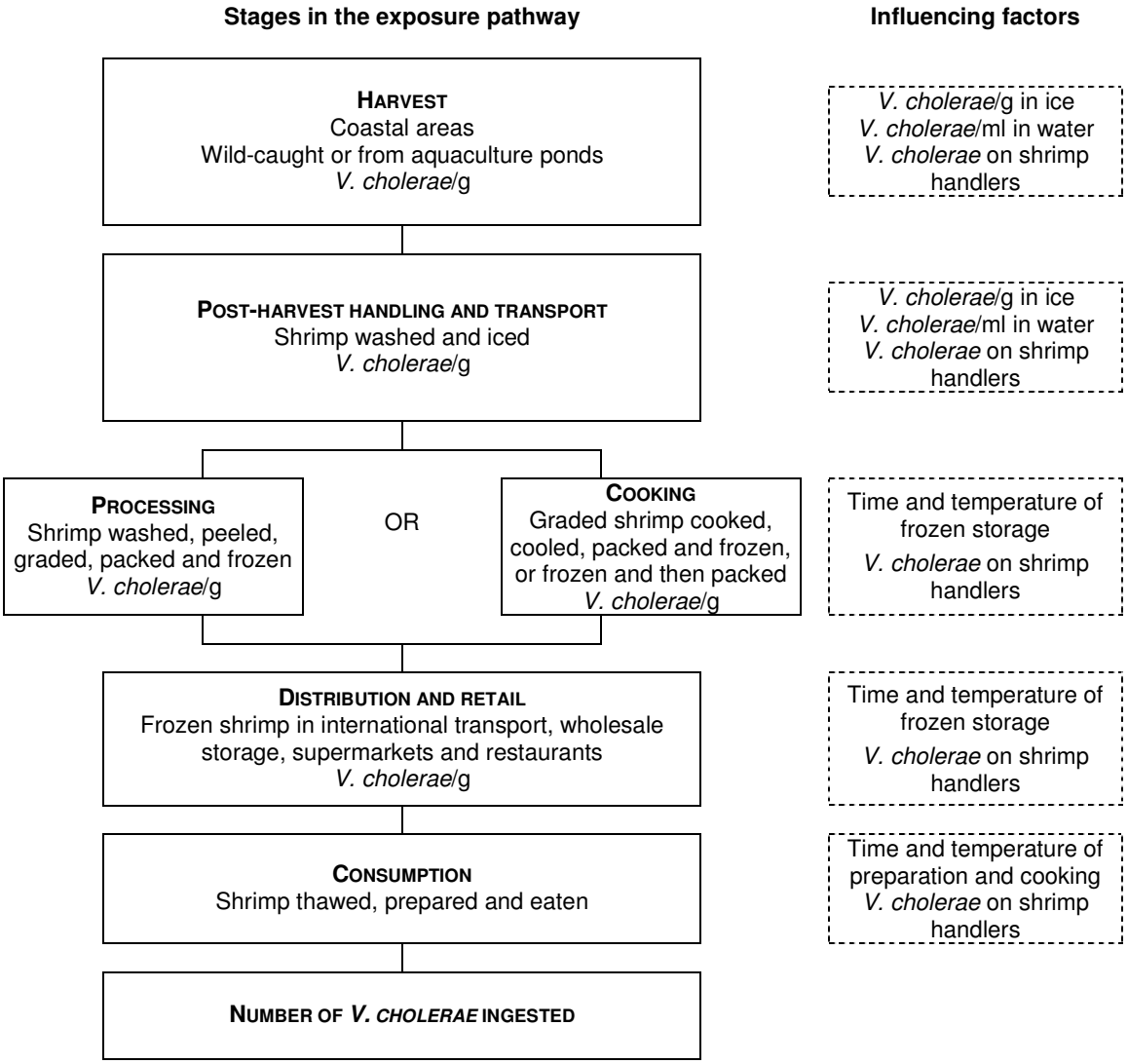

Figure 1. Production-to-consumption pathway for exposure assessment of *V. cholerae* in warm-water shrimp harvested and processed for international markets.

4.2.2. Harvest, post-harvest handling and transport

Marine shrimp harvested by trawling are separated from the by-catch by manual sorting, and then iced on board. Ice is generally produced using potable water in coastal ice plants and

carried on board in insulated containers. During on-board handling, contamination with *V. cholerae* is possible if the person handling shrimp and ice is a carrier of *V. cholerae* O1, or if the ice has become contaminated with choleragenic *V. cholerae*. However, the use of potable water – and in many cases the implementation of a HACCP system (as required for products exported to many countries e.g. the United States of America and countries of the European Union) in the production and handling of the ice – minimizes the opportunity for faecal contamination of ice.

In cholera-endemic areas, asymptomatic carriers play an important role in transmission of the pathogen. In fact, for water stored in households, contamination through the hand contact of carriers has been observed as a route for transmission of cholera. This is borne out by studies conducted in Calcutta, India (Deb et al., 1986). A series of studies was conducted in which sanitary wells were installed in communities and the effect on incidence of cholera and other enteric diseases measured. The results generally indicated no reduction in the incidence of cholera or diarrhoea in users of sanitary wells. The major reason for this appears to be that water is rarely ingested directly from source, but is stored in the household in a variety of ways. These studies suggest that asymptomatic carriers may contaminate waters by putting their hands or utensils in water that is stored in wide-mouthed containers; introduction of narrow-necked earthenware vessels for storing water reduced the *V. cholerae* O1 infection rate by 75% (Deb et al., 1986). Thus, contamination via the hands of shrimp handlers is a possible route. However, where personal hygiene and other hygienic conditions are controlled by the implementation of GHPs and a HACCP system in shrimp processing, the likelihood of faecal contamination of shrimp via fingers becomes very low.

In the case of cultured shrimp, faecal contamination with choleragenic *V. cholerae* may occur during harvest and handling before the shrimp is washed and chilled. The likelihood and level of such contamination is unknown, although the implementation of GHPs and HACCP along the chain should mean that this is low. Control points include dipping or washing in tap water, icing, and packing in plastic crates for transport by truck to the processing plant. The process of dipping or washing may reduce the level of *V. cholerae* in shrimp, as shown by Dinesh (1991), who demonstrated that a one-log reduction in counts of *V. cholerae* was brought about when whole shrimp spiked with the organism were dipped or washed in tap water.

4.2.3. Processing and cooking

If contamination of shrimp with choleragenic *V. cholerae* were to occur during post-harvest handling, factors influencing the level of the organism include time and temperature during handling, processing and storage. Time-temperature distributions and the effects on densities of choleragenic *V. cholerae* are presented in Table 5. The study of Kolvin and Roberts (1982) indicates that *V. cholerae* O1 does not multiply in raw shrimp. Further, the temperature of iced shrimp during transport would be <10°C, at which temperature *V. cholerae* O1 does not multiply. The temperature range for growth of *V. cholerae* O1 and O139 is 10–43°C (ICMSF, 1996). Studies in India (I. Karunasagar, personal communication, 2002) confirm that *V. cholerae* O1 does not multiply in raw shrimp stored in ice. This study was conducted by contaminating the surface of shrimp with *V. cholerae* O1 at a level of 10^8 cfu/g, storing the shrimp in ice and estimating the numbers of *V. cholerae* by direct plating on non-selective agar followed by hybridization of the colonies with a *ctx* gene DNA probe. Over a 6-hour period, a

Table 5. Time and temperature distributions and the effect on levels of choleragenic *V. cholerae* in warm-water shrimp, both wild-caught and from aquaculture.

Processing step	Temp. range	Time range	Effect on population of *V. cholerae* O1	Source
HARVEST				
Handling in period before icing				
Aquaculture shrimp	15–35°C	0–1 hours	No effect	(a) (b)
Wild-caught shrimp	10–30°C	0–3 hours	0–1 log increase	
WASHING				
Washing and icing of aquaculture shrimp	0–7°C	1–4 hours		
Washing in seawater of wild-caught shrimp	0–30°C	1–4 hours	1 log reduction	(c)
ICING				
Icing during transport (including aboard fishing vessel for wild-caught shrimp) to processor	0–7°C	2–16 hours (aquaculture) 2–48 hours (wild-caught)	2–3 log reduction	(d)
WATER USE				
Water use during handling at processing plant	4–10°C	1–3 hours	No effect	(a) (b)
TEMPERATURE				
Temperature during processing before freezing	4–10°C	2–8 hours	No effect	(a) (b)
COOKING				
Cooking at processing plant	>90°C	0.5–1.0 minute (This is the holding time at >90°C)	>6 log reduction	(e) (f)
FREEZING				
Freezing of cooked and raw products, storage, and shipment time	-12° to -20°C	15–60 days	2–6 log reduction	(g) (h)

SOURCES: (a) Industry data for time and temperature from M/S Sterling Seafoods, Mangalore, India, personal communication, 2002. (b) Multiplication from Kolvin and Roberts, 1982. (c) Dinesh, 1991. (d) Karunasagar, India, personal communication, 2002. (e) Based on industry data on total plate count from M/S Sterling Foods, Mangalore, India, personal communication, 2002. (f) In shrimp homogenate, $D_{82.2} = 0.28$ (Hinton and Grodner, 1985). (g) INFOFISH, personal communication, for shipment time; Reilly and Hackney, 1985. (h) Survival in frozen shrimp from Nascumento et al., 1998.

3-log reduction was observed and this level was maintained for 48 hours. This study suggests that transport of shrimp in ice is likely to bring about a reduction in density of choleragenic *V. cholerae*.

Irrespective of country of origin, warm-water shrimp is a high-value commodity. When intended for export it is processed in facilities that meet sanitary requirements for GHPs, GMPs and HACCP. Once shrimp arrives in a processing plant, it is peeled manually or by machine, then washed, graded, processed (e.g. deheading, gutting) and, in some cases cooked, before being packed for freezing.

Cooking is undertaken for several reasons, most important of which are customer specifications or the prevention of melanosis (black spot formation), which can occur in the head during chilled storage. In Australia, Winkel (1997) studied the effect of cooking on black spot formation and organoleptic quality of *Penaeus monodon* (Black tiger shrimp). Winkel established that a core temperature of 75°C was sufficient to cook the flesh, but that prevention of black spot required a core temperature of 85°C. The time required to reach a 75°C core temperature was related to the size of shrimp: almost 4 minutes for "large" (50–65 g) shrimp and 1.5 minutes for "small" (25–30 g) shrimp.

As part of an Australian code of practice for farmed shrimps, Sumner (1997) observed shrimp processing at six processing plants. Operators lowered each batch of shrimp into "boiling" water (ca 98°C) in a proportion of around 5:1 (water:shrimp), a procedure which

lowered the water temperature to around 92°C. The source of heat – usually a gas-fired ring – was then maximized and the water quickly brought to "boiling" (ca 98°C), at which time the operator activated the timing device for the process. Since overcooking leads to poor organoleptic quality and to weight loss, cooking time is important. Depending on size, shrimp were cooked for between 0.5 minute ("small") and 1.0 minute ("large"), then immediately plunged into an ice-water slurry to bring an end to cooking. A similar industrial cooking process has been described by I. Karunasagar (personal communication, 2002) at a plant in Mangalore, India (see Table 5).

Since contamination with *V. cholerae* is likely to be external, the site of greatest microbiological concern for shrimp is the carapace. From the foregoing, it is clear that the site of microbiological concern receives a highly lethal heat treatment. For example, Hinton and Grodner (1982) cite $D_{82} = 0.24$ for *V. cholerae* in oyster homogenate, and $D_{82} = 0.28$ in shrimp homogenate (Hinton and Grodner, 1985). Thus, with at least 60 seconds at >90°C, the lethality is greater than 6 log units. It is opportune that the short cooking time (>90°C for 0.5–1.0 minute) equates with a highly lethal outcome at the site of microbiological concern.

The objective post-cooking is to reduce the temperature of the shrimp to as low as possible and as quickly as possible, and to minimize the length of time at which the shrimp are at a temperature suitable for microbial growth. In many plants this involves quickly moving the shrimp (in most plants this is now mechanized) into gyro-freezers (or similar) to produce individually quick frozen (IQF) shrimp (H. Lupin, personal communication, 2005). The opportunity for post-cooking re-contamination (from water or ice, or both or shrimp handlers) of shrimp is minimised when processed in accordance with a HACCP system combined with GHPs, GMPs and SSOPs.

During freezing, it can be expected that there will be a reduction in the level of *V. cholerae*. Nascumento et al. (1998) noted that when whole shrimp were dipped in a solution of *V. cholerae* O1 and then frozen at -20°C, numbers were reduced from about 7-log units to less than 1-log unit in 36–38 days; inactivation was more rapid in shrimp without a shell (30 days). However, this study has several limitations because direct plating on TCBS agar was used for bacterial enumeration and stressed cells, which might be resuscitated during pre-enrichment in alkaline peptone water (APW), may not be capable of growth on TCBS following direct plating (Nascumento et al., 1998). Thus, even though frozen shrimp may harbour low levels of *V. cholerae* O1, the numbers would further decline during frozen storage. According to industry sources in India (M/S Sterling Seafoods, personal communication, 2002), the time interval between packing and the item reaching the port-of-entry in the importing country is usually more than 30 days, with INFOFISH (www.infofish.org) data indicating a range of 15 to 56 days. Thus, a low prevalence and density of choleragenic *V. cholerae* in harvested shrimp during handling and processing, followed by significant reductions during freezing, explains the low prevalence of choleragenic *V. cholerae* O1 and O139 in frozen shrimp reported at port-of-entry testing, as illustrated in Table 6.

The foregoing traces a processing continuum in which, at various stages, there is progressive inactivation of *V. cholerae*, particularly when product is held under refrigeration (iced or frozen), of the order of 5–6 log units. Cooking leads to additional inactivation of the order of 6 log units.

Table 6. Number and country of origin of imported shrimp samples tested for choleragenic *V. cholerae* O1 and O139 in Denmark, Japan and the United States of America.

Country of origin	1995	1996	1997	1998	1999	2000
JAPAN IMPORTS						
Iran	1	4	4	7	7	12
India	1 452[a]	1 495	873	718	563	561
Indonesia	1 001	969	710	676	499	335
Cambodia	0	0	2	0	10	3
Sri Lanka	13	0	22	83	56	102
Thailand	849	780	693	471	395	350
Nepal	0	0	2	0	0	0
Bangladesh	214	151	53	46	104	58
Philippines	324	200	263	215	172	64
Viet Nam	558	488	419	408	313	395
Malaysia	75	76	62	66	45	41
Myanmar	126	162	102	82	100	61
Taiwan	57	34	22	10	7	3
Argentina	30	32	12	10	0	0
Ecuador	147	67	48	55	46	11
Guyana	8	4	2	0	0	0
Guatemala	0	0	0	0	2	0
Colombia	20	18	14	8	9	15
Surinam	34	24	32	23	19	23
Chile	8	0	0	0	0	0
Nicaragua	6	16	10	0	0	0
Panama	0	4	2	0	0	0
Brazil	85	122	47	40	30	24
Venezuela	2	2	1	0	0	0
Belize	2	2	0	0	0	0
Peru	0	0	2	0	0	2
Honduras	6	2	0	0	0	0
Mexico	70	108	10	78	32	19
Ghana	0	0	0	0	2	0
Guinea	2	0	0	0	0	0
Senegal	0	6	2	0	0	0
Tanzania	0	0	0	0	5	0
Nigeria	0	2	0	0	0	0
Madagascar	0	0	0	0	16	17
Mauritania	0	0	0	0	0	0
Mozambique	20	18	18	9	13	28
South Africa	0	0	0	0	0	5
UNITED STATES OF AMERICA IMPORTS						
India						148
Thailand						5
Ecuador						16
Venezuela						2
Chile						14
Mexico						10
DENMARK imports [b]	752					
Other countries imports	2	6	0	0	0	0
TOTAL	5 864	4 792	3 427	3 005	2 445	2 324

NOTES: (a) 2 samples imported into Japan from India in 1995 tested positive for choleragenic *V. cholerae* O1. The two positive samples were of washed, size-selected, de-headed, frozen prawn, with no cooking steps.
(b) A total of 3 555 tonne of warm-water shrimp was imported into Denmark between December 1994 and July 1995 (790 tonne of raw frozen products and 2 765 tonne of cooked frozen products). Products were imported from a total of 10 countries, with raw products predominantly from Thailand (52%) and Bangladesh (37%), and cooked products from Viet Nam (24%), Thailand (24%), Chile (17%) and Bangladesh (14%).

Taken together, inactivation during processing and frozen storage provides an explanation for the lack of any documented involvement of internationally traded shrimp in outbreaks of cholera in shrimp importing countries. The maintenance of high hygienic standards by shrimp exporters was amply demonstrated during the Peruvian cholera epidemic in 1991. DePaola et al. (1993) showed that, while choleragenic *V. cholerae* O1 was present in all five samples of raw seafood collected from street vendors in Lima and Callao, it could be isolated from only 1 out of 1011 samples of seafood destined for export. This shows that, even during a large epidemic, contamination of seafood with *V. cholerae* O1 can be prevented through the adoption of strict hygienic measures during handling and processing of shrimp.

As part of the present assessment, a number of countries were contacted to obtain information on detection of choleragenic *V. cholerae* O1 and O139 in imported shrimp products. Data were received on findings in warm-water shrimp imported into Denmark, Japan and the United States of America (Table 6). This data was collected using an enrichment procedure at 35°C, although a recent study in Malaysia (Chen et al., 2004; Elhadi et al., 2004) suggests this procedure might not detect all *V. cholerae* present. While some data reported to the EU by member countries were available for *V. cholerae*, such reports typically did not include information about the serovars and presence of the cholera toxin gene in the *V. cholerae* strains isolated, and so were not used in this risk assessment. The vast majority of testing data shown in Table 6 originate from Japan, with more than 20 000 shrimp samples tested from 1995 to 2000 (Office of Quarantine Administration, Department of Food Safety, Ministry of Health, Labour and Welfare, Japan). Data for the United States of America were obtained from the Food and Drug Administration (FDA) and included only the year 2000, in which a total of 181 samples were tested; more than 80% of the samples (148 samples) tested in the United States of America originated from India. Of 3 555 tonne of warm-water shrimp, mostly cultured, imported into Denmark from December 1994 to July 1995, choleragenic *V. cholerae* O1 was not detected in any of 752 samples analysed (Dalsgaard et al., 1996) (Table 6). The imported warm-water shrimp samples that were analysed in Denmark originated mainly from Bangladesh, Chile, Thailand and Viet Nam (Dalsgaard et al., 1996). In Thailand, the Fish Inspection and Quality Control Division of the Department of Fisheries routinely examines frozen shrimp for the presence of *V. cholerae*. During 2001, 1 319 samples were analysed, and during 2002, 1 064 samples were tested. Choleragenic *V. cholerae* was not detected in any of the samples (data submitted to FAO by Department of Fisheries, Thailand). In 2005, shrimp imported into Europe tested positive for *V. cholerae* but further analysis indicated that these were non-toxigenic strains (Appendix C).

Of the total of 21 857 warm-water shrimp samples tested, only 2 samples imported into Japan from India in 1995 tested positive for choleragenic *V. cholerae* O1 (Table 6). The pathogen was not detected in smaller sample sets from Denmark and the United States of America. In the exposure assessment, sampling and detection data from all years and countries were pooled and used to develop the expected distribution of choleragenic *V. cholerae* O1 and O139 densities in imported shrimp. Methods for isolation of *V. cholerae* in Denmark, Japan and the United States of America, although not identical, were comparable.

4.2.4. Distribution and retail

Since the product is stored under frozen or refrigerated conditions, the retail market in importing countries provides little opportunity for contamination or multiplication of *V. cholerae* O1 in

shrimp,. This is supported by epidemiological data from countries such as Japan and the United States of America, where shrimp consumption is high (Table 7) and reported numbers of domestically acquired cholera cases are absent or very low (Table 8). There are no reports of either outbreaks or sporadic cases of cholera associated with imported shrimp.

Table 7. Number of servings (estimated) of warm-water shrimp consumed in selected countries.

		1995	1996	1997	1998	1999	2000
USA	Volume (tonne $\times 10^3$)	270.9	264.2	294.1	315.4	331.7	345.1
	Number of servings ($\times 10^7$)	98	96	107	115	121	125
Japan	Volume (tonne $\times 10^3$)	265.4	260.1	245.8	218.2	225.2	235.3
	Number of servings ($\times 10^7$)	96.5	94.6	89.4	79.3	81.9	85.5
Italy	Volume (tonne $\times 10^3$)	19.2	20.8	17.3	21.2	19.2	21.0
	Number of servings ($\times 10^7$)	7.0	7.6	6.3	7.7	7.0	7.6
Spain	Volume (tonne $\times 10^3$)	61.2	62.0	62.1	74.4	69.4	75.9
	Number of servings ($\times 10^7$)	22.3	22.5	22.6	27.1	25.2	27.6
Germany	Volume (tonne $\times 10^3$)	13.3	12.0	14.3	14.5	13.8	15.6
	Number of servings ($\times 10^7$)	4.8	4.4	5.2	5.3	5.0	5.7
UK	Volume (tonne $\times 10^3$)	22.5	22.2	22.0	24.6	25.5	30.0
	Number of servings ($\times 10^7$)	8.1	8.0	8.0	8.9	9.2	10.9
France	Volume (tonne $\times 10^3$)	42.9	46.4	45.1	51.6	50.6	46.5
	Number of servings ($\times 10^7$)	15.6	16.9	16.4	18.8	18.4	16.9

NOTE: Number of servings calculated from total weight of warm-water shrimp imported (g) divided by the estimated average serving size (275 g).

Table 8. Cases of cholera notified to WHO from major shrimp importing countries.

Country	Cholera cases					
	1995	1996	1997	1998	1999	2000
Japan	311 (295i)[a]	39 (35i)	89 (55i)	60 (57i)	40	34 (32i)
USA	19 (19i)	3[b]	4 (4i)	15 (15i)	6 (6i)	4 (1i)
Spain	6 (6i)	1 (1i)	–	–	–	1 (1i)
France	5 (5i)	6 (6i)	3 (3i)	2 (2i)	–	–
Denmark	3 (3i)	–	–	–	–	–
Italy	1 (1i)	–	–	2	–	–
The Netherlands	9 (9i)	3 (3i)	2 (2i)	4 (4i)	2 (2i)	–
United Kingdom	10 (10i)	13 (13i)	6 (6i)	18 (18i)	–	33 (33i)
Canada	7 (7i)	2 (2i)	–	2 (2i)	–	5 (2i)
Hong Kong[c]	6 (4i)	4(1i)	14	71 (38i)	18 (11i)	9 (3i)
Germany[d]	1 (1i)	–	2i	5 (5i)	3 (3i)	2 (2i)

NOTES: (a) i = imported cases (WHO, 2001). (b) 1 case in Guam and 1 case in Saipan reported by CDC. (c) From 1997, Hong Kong Special Administrative Region of China. (d) Not in top ten importing countries but considered in the risk assessment due to the availability of relevant data.

SOURCE: WHO Weekly Epidemiological Record, various dates.

As discussed in the previous section, contamination with *V. cholerae* O1 may occur during retailing of shrimp in domestic markets in endemic areas of cholera and in developing countries experiencing cholera outbreaks. The data of De Paola et al. (1993) from the Peruvian cholera epidemic showed high levels of contamination (100%) in a small number of raw seafood samples from street vendors. However, choleragenic *V. cholerae* O1 was not isolated during increased sampling and analyses of imported shrimp from South American countries during the cholera epidemic of the early 1990s (DePaola et al., 1993). There are no data on the levels of *V. cholerae* O1 found in raw shrimp from street vendors, and therefore it is unknown if *V. cholerae* O1 would multiply to infectious levels in shrimp during retailing conditions in developing countries.

4.2.5. Consumption

Storage and preparation in the home and food services

As a generalization, in importing countries, cold chain systems linking distribution, home and food service prevent temperature abuse. A survey by Audits International (2000) indicated that only 1.5% of home refrigerators operated above 10°C, the minimum temperature for growth of *V. cholerae*. Predicted levels of the organism based on time-temperature relations through the food chain (Table 5) support this contention that the cold chain prevents growth of the organism. Cross-contamination of other food, while possible, is unlikely to progress to an infectious dose unless overt temperature-time abuse occurs.

Volumes of imported shrimp consumed in selected countries

Due to a lack of specific information on the amount of imported shrimp consumed in selected countries, alternative approaches were used to estimate the amount of shrimp consumed, using FAO data on warm-water shrimp imports (Table 9). Shrimp import data from 1995 to 2000 were available for France, Germany, Italy, Japan, Spain, the United Kingdom and the United States of America (FAO-Globefish, 2002) and was the basis for selecting those countries. The United States of America and Japan are the leading importers of warm-water shrimp, followed by a number of European countries. Since the risk assessment is limited to consumption of warm-water shrimp, only data on imports from tropical countries are listed. It was not possible, however, to obtain information about the volumes imported of the various product formats, namely cooked, peeled, shell-on, etc..

Table 9. Warm-water shrimp imports (tonne $\times 10^3$).

Importing country	1995	1996	1997	1998	1999	2000
Spain	61.2	62.0	62.1	74.4	69.4	75.9
Italy	19.2	20.8	17.3	21.2	19.2	21.0
Germany	13.3	12.0	14.3	14.5	13.8	15.6
USA	270.9	264.2	294.1	315.4	331.7	345.1
Japan	265.4	260.1	245.8	218.2	225.2	235.3
France	42.9	46.4	45.1	51.6	50.6	46.5
UK	22.5	22.2	22.0	24.6	25.5	30.7

Shrimp portion sizes and estimated number of servings

The total volumes of imported warm-water shrimp, i.e. volume of shrimp with known and unknown ("other") country of origin, were used to estimate the total annual numbers of servings

consumed in each importing country. The total imported volumes were used to calculate the assumed edible weight in grams (Table 7).

The distribution of meal sizes was created without the benefit of data and therefore the mean serving size was assumed to be 275 g/meal, though, in reality, the edible portion will be <275 g after the carapace and cephalothorax have been removed prior to consumption. This assumption was made on the basis of an average serving of shrimp consisting of 10 small shrimp, described as weighing between 25 and 30 g (average 27.5 g) (Winkel, 1997) or 5 large shrimp, described as weighing 50–65 g (Winkel, 1997).

Format of shrimp at consumption

In international trade, shrimp are marketed frozen in both the raw and cooked forms. In the cooked form, shrimp are consumed without further heat treatment. Typically, raw shrimp are cooked before consumption, though, with the growing popularity of sushi and sashimi, a proportion may be eaten without further heat treatment; this proportion might be expected to be higher in Japan than in the other countries selected in this risk assessment. For the purposes of the present assessment it was assumed that 10% of warm-water shrimp would be consumed raw and 90% consumed after cooking (either during production or during meal preparation). However, the proportion assumed to be consumed raw is conservative and probably an overestimate, as one report from Japan estimated that less than 1% of shrimp were consumed raw (MAFF, 2001).

4.3. Assumptions regarding exposure assessment

As there are gaps in our knowledge base, a number of assumptions were made in developing the exposure assessment model. The primary assumptions are:

- *V. cholerae* are distributed homogeneously in shrimp.
- The mean serving size is 275 g.
- The use of volumes of warm-water shrimp imported provides a realistic basis for the amount of shrimp consumed.
- Cooked shrimp account for 90% of product consumed, while 10% of the product was assumed to be consumed raw.

5. Hazard characterization

5.1. Pathogen, host, and food matrix factors

5.1.1. Characteristics of the pathogen

V. cholerae O1 and O139 are well known for causing the gastrointestinal illness known as cholera, which in its severe form, cholera gravis, is an illness characterized by the passage of voluminous stools leading to dehydration. If untreated, the resulting dehydration can lead to hypovolemic shock and the death of the patient within 18 hours to several days, or sooner in extreme cases, of onset of symptoms (Bennish, 1994). The case-fatality rate in untreated cases may reach 30-50%. However, treatment is straight forward and if applied appropriately the case-fatality rate is less that 1% (WHO, 2004). Only the choleragenic strains of *V. cholerae* O1 and O139 are considered in this analysis; however, the main focus of the risk assessment is *V. cholerae* O1, since very limited information was available for *V. cholerae* O139, particularly in relation to the exposure assessment.

V. cholerae is sensitive to acid and therefore must successfully pass the acid barrier of the stomach in order to cause infection. Choleragenic *V. cholerae* are known to have several genetic factors related to virulence. In order to establish itself and multiply in the human small intestine, choleragenic *V. cholerae* O1 and O139 have one or more adherence factors that enable them to attach to the microvilli or intestinal epithelial cells (Kaper, Morris and Levine, 1995). The *ctx* operon is, however, the primary factor associated with choleragenicity (see also Section 3.1.1). The *ctx* operon codes for cholera toxin (CT), which is made up of an A and B subunit and is responsible for the symptoms of cholera. These include the disruption of ion transport, with the subsequent loss of water and electrolytes leading to severe diarrhoea.

This toxin is secreted by the choleragenic *V. cholerae* O1 and O139 strains. The O1 serogroup can be classified into three main sub-groups: Ogawa, Inaba and Hikojima. Strains may be subclassified into two biotypes: Classical and El Tor (Kaper, Morris and Levine, 1995). Genetic studies have shown that the *V. cholerae* O139 choleragenic strain has evolved from an El Tor biotype (Faruque et al., 2003).

5.1.2. Characteristics of the host

Health factors

The host immune system is the critical defence mechanism against cholera. However, infection with cholera can result in a range of responses, from severe and life threatening diarrhoea to mild or unapparent infections. In endemic areas, for example, only a minority (20–40%) of infections with *V. cholerae* O1 El Tor results in any illness (Bart et al., 1970; Shahid et al., 1984). Studies from rural Bangladesh indicated that only 11% of persons exposed to *V. cholerae* O1 in household water developed infection, and only half of the infected individuals developed illness (Spira et al., 1980). The level of contamination in water ranged from 1–500 cfu/100 ml. Glass and Black (1992) estimated that 10^2–10^3 organisms are likely to result in illness, though the infectious dose determined by human volunteer experiments is much higher. The reasons for

these differences are not completely clear. However, prior immunologic experience is certainly an important factor, and reports of much higher attack rates in children compared with adults in cholera-endemic areas support this (Glass et al., 1982). Another factor may be differences in gastric acidity. As gastric acid is an important defence mechanism against cholera, low acid production can lead to increased susceptibility (Nalin et al., 1978; Van Loon et al., 1990).

Recurrent infections of cholera are rare, and volunteer studies have shown that that clinical cholera infection confers 90 to 100% protection against subsequent re-challenge with choleragenic *V. cholerae* (Cash et al., 1974; Levine et al., 1979, 1981; Levine, 1980). In addition, epidemiological studies in endemic areas have indicated that immunity follows an initial natural cholera infection (Glass et al., 1982).

Pregnant women appear to experience a more severe form of the disease than non-pregnant women. In addition, foetal loss is high, with one report indicating a foetal death rate of 50% among women in their third trimester of pregnancy who developed severe dehydration from cholera (Hirschhorn, Chowdhury and Lindenbaum, 1969).

Demographic and socioeconomic factors

A number of demographic and socioeconomic factors, such as age, gender, nutritional status, social status, economic status and travel abroad, all play a role in susceptibility to choleragenic *V. cholera.* Sanitation and nutrition are particularly important factors.

It has become clear with time that good sanitation practices and good hygienic practices largely prevent this disease. In addition, *V. cholerae* strains are relatively susceptible to inactivation by cooking (Hinton and Grodner, 1982, 1985). Most of the risk associated with choleragenic *V. cholerae* in food comes from cross-contamination (from food handlers, water or raw food), particularly for foods that will receive no further heat treatment during meal preparation.

V. cholerae infection is known to be more severe in individuals suffering from malnutrition. Hypochlorhydria associated with malnutrition, B_{12} deficiency and gastritis predispose to the development of cholera. However, undernutrition does not seem to be associated with increased risk (Richardson, 1994).

In cholera-endemic areas, children 2–15 years are considered most susceptible to cholera when this group experiences initial infection (Glass et al., 1982). The symptoms of first infections are severe, but rarely are people hospitalized a second time for the disease, suggesting that immunity is long lasting and protects against severe illness. Breastfeeding appears to be an important factor in reducing susceptibility to cholera among infants and young children. One study indicated 70% reduction in the risk of severe cholera among breast-fed children (Clemens et al., 1990). In cholera-endemic areas, women of childbearing age (15–35) are commonly infected. In developed countries where hygienic standards are high, all age groups are equally susceptible (Kaper, Morris and Levine, 1995). Most cases in countries where high hygienic standards exist are imported cases, in that exposure to *V. cholerae* occurred while travelling in another country.

Genetic factors

Among host susceptibility factors, notable is the association between cholera and blood group. Barua and Paguio (1977) and Chaudhuri and De (1977) noted that the incidence of cholera in patients with blood group A was lower than that in the general population, while incidence in those with blood type O was significantly higher. The likelihood of *V. cholerae* infection

progressing to the severe form, cholera gravis, appears to be related to the individual's ABO blood group (Levine et al., 1979). Thus, individuals with blood group O are more likely to exhibit severe diarrhoea. In terms of genetic factors, there is a hypothesis that those heterozygous for the cystic fibrosis allele are apparently less susceptible to severe cases of cholera (Rodman and Zamudio, 1991).

5.1.3. Characteristics of the food matrix

While choleragenic *V. cholerae* O1 ingested with food is likely to be protected from gastric acid, human volunteer studies have produced mixed results. In one study, human volunteers ingested 10^6 *V. cholerae* O1 El Tor with 2 g of sodium bicarbonate ($NaHCO_3$) in 300 ml water or with a meal of fish, rice, milk and custard (Levine et al., 1981). Volunteers who ingested *V. cholerae* with water alone did not become infected, but those who ingested the organism in a meal had cholera of similar severity and attack rate (Figure 2) to those who had buffered gastric acidity with $NaHCO_3$ (Levine et al., 1981). By contrast, experiments by Cash et al. (1974) showed different results (see Section 5.4).

Fat and salt content

Fat and salt content are probably not relevant in the determination of risk with respect to choleragenic *V. cholerae* O1 and O139. However, while the fat content of a matrix may be relevant with respect to the increase of effective dose of pathogens through protection of the organisms in micelles during gastric passage, there is insufficient evidence to model the degree of increased survival.

pH and water activity

Choleragenic *V cholerae* appear to be relatively sensitive to both low pH and dehydration. The pH sensitivity of *V. cholerae* is illustrated by the epidemiological data of St Louis et al. (1990), who observed that, in an epidemic in Guinea, West Africa, the cholera patients were more likely to have eaten left-over peanut sauce (pH 6.0) but less likely to have eaten tomato sauce (acid). This was further confirmed by laboratory studies in which *V. cholerae* multiplied rapidly in peanut sauce but not in more acidic tomato sauce.

V. cholerae O1 are extremely sensitive to an acidic environment (Dalsgaard et al., 1997). In gastric juice with pH <2.4, *V. cholerae* O1 were inactivated rapidly (Nalin et al., 1978; Levine et al., 1984). Since *V. cholerae* O1 are transmitted via the oral route only, the organisms must pass through the gastric acid environment of the stomach to colonize the intestine. In normochlorhydric adult volunteers, doses of up to 10^{11} pathogenic *V. cholerae* O1 given without buffer or food did not reliably cause illness, whereas doses of 10^4–10^8 organisms given with 2 g of $NaHCO_3$ resulted in diarrhoea in 90% of individuals (Cash et al., 1974). The characteristics of illness in individuals with 10^6 organisms given with 2 g of $NaHCO_3$ were similar to that of cholera. In another volunteer study, doses of 10^5, 10^4 and 10^3 organisms resulted in a 60% attack rate, although the diarrhoeal illness at the two lower doses was milder and appeared to have longer incubation periods (Levine et al., 1981). The dose-response study of Levine et al., (1981) is presented in Table 10.

Because of the nature of most foods associated with the unintended consumption of *V. cholerae*, pH and water activity are probably not relevant in modelling survival of *V. cholerae* in raw seafood. However, these parameters may be relevant in modelling the growth of *V. cholerae* in other foods as a result of cross-contamination.

Table 10. Clinical response of healthy North American volunteers to various doses of *V. cholerae* El Tor Inaba Strain N 16961.

Dose[a]	Clinical attack rate	Mean incubation time (hours)	Mean diarrhoeal stool volume (litre) per ill volunteer (range)	Mean no. of loose stools per ill volunteer (range)
10^6	9/10[b]	25.5	3.2 (0.4–13.1)	12.9 (2–39)
10^5	3/5	18.0	3.1 (0.4–3.7)	15.0 (9–21)
10^4	4/5	36.5	1.1 (0.6–1.5)	6.5 (4–10)
10^3	4/6	33.3	0.9 (0.4–1.9)	5.8

NOTES: (a) Volunteers ingested 2 g sodium bicarbonate prior to ingesting inoculum.
(b) Number ill/number of volunteers challenged.
SOURCE: Levine et al., 1981.

5.2. Public health outcomes

5.2.1. Manifestations of disease

When illness occurs, *V. cholerae* O1 and O139 cause mild to severe gastrointestinal illness and may bring about patient dehydration leading to death. Common symptoms include profuse watery diarrhoea, anorexia and abdominal discomfort. In cholera gravis, the rate of diarrhoea may quickly reach 500–1000 ml/hour, leading rapidly to tachycardia, hypotension, and vascular collapse due to dehydration (Kaper, Morris and Levine, 1995). About 20% of those who are infected develop acute, watery diarrhoea and 10 to 20 % of these individuals go on to develop severe watery diarrhoea with vomiting (WHO, 2004).

5.2.2. Rationale for the biological end points modelled

Severe gastrointestinal illness was modelled as the endpoint in this risk assessment, as this is the more common outcome of cholera infection in countries importing warm-water shrimp. There are also human volunteer data available to correlate dose with likelihood of diarrhoea and thus facilitate the development of a dose-response model. These human volunteer studies were carried out in countries that import shrimp and were those considered in this risk assessment.

5.3. Number of cholera cases reported to WHO by shrimp importing countries

Cases of cholera reported to WHO by shrimp-importing countries and used in this assessment were listed in Table 8. Cholera is one of the diseases requiring notification to WHO according to the International Health Regulation. It is worth noting that the United Kingdom (Adak, Long and O'Brien, 2002) and the United States of America (Mead et al., 1999) estimate that 50% of all cholera cases are reported, which is high compared with the level of reporting of some other gastrointestinal illnesses, such as non-typhoidal salmonellosis and campylobacteriosis, for which actual cases are estimated to be 38 times more than reported cases (Mead et al., 1999).

In the available documentation, none of the cholera cases reported has been associated with consumption of imported warm-water shrimp. Except for a few, all cholera cases in the United States of America and European countries were overseas-acquired. In 1998, Italy reported two domestically-acquired cholera cases. Epidemiological evidence indicated that both cases had consumed a seafood salad produced by an Italian manufacturer using cooked frozen seafood imported from different European producers. Microbiological assays of the salad were negative

for *V. cholerae* O1 (EC, 1998). The United States of America has an endemic focus of *V. cholerae* in Gulf Coast waters and has experienced sporadic cases and small clusters of cholera related to the domestic consumption of contaminated seafood from those waters (Blake et al., 1980; Blake et al., 1983). Japan has reported an increasing number of apparent domestically-acquired cholera cases (IASR, 1998). The reason(s) for this increase is unknown, cases typically being sporadic with no known aetiology.

5.4. Dose-response relationship

Dose-response relationships can be developed from epidemiological investigations of outbreaks and sporadic case series, human feeding trials or animal models of a particular pathogen and related (surrogate) pathogens. In this instance, human feeding trial data were available for *V. cholerae* and were used in the development of the dose-response curve.

5.4.1 Summary of available data

There are numerous studies and references in the literature to the infectious dose of choleragenic *V. cholerae*. The most commonly reported infectious dose is approximately 10^6 organisms or more (Levine et al., 1981; Tauxe et al., 1994b; Health Canada, 2001; FDA, 2003). While, as indicated in Section 5.1, there are numerous other factors that influence whether or not a person becomes ill after ingestion of choleragenic *V. cholerae,* this estimate was used in both the qualitative and semi-quantitative risk characterizations described in Section 6. A number of human volunteer studies are available for choleragenic *V. cholerae*. Although these are between 15 and 30 years old, they are the best data available in terms of providing an insight into the dose response of choleragenic *V. cholerae.* These data have been extensively considered and used as a basis to develop a dose-response model, as described below. It is worth noting that these human volunteer feeding trials were undertaken using healthy volunteers.

Probability of illness given exposure

Human volunteer data are available for the Classical and El Tor biotypes and Inaba and Ogawa serogroups of *V. cholerae* O1. Cash et al. (1974) studied Classical Inaba and Ogawa strains, while Levine et al. (1988) and Black et al. (1987) studied El Tor Inaba and Ogawa strains. The results from these studies are shown in the dose-response curve presented in Figure 2. As noted above, volunteer data were also available for *V. cholerae* O139.

Probability of sequellae given illness

Many choleragenic *V. cholerae* O1 and O139 infections result in the serious condition called cholera gravis, which can be life threatening. There are no specific sequellae associated with the severe form of illness other than the risk of death.

Probability of secondary and tertiary transmission

While illness due to choleragenic *V. cholerae* O1 and O139 is observed to occur in families, it is thought that a common source of primary infection, rather than secondary transmission, is the more likely mode of transmission (Glass and Black, 1992). While there is anecdotal indication that direct person-to-person transmission may occur, it has never been demonstrated by rigorous scientific study (Mintz, Popovic and Blake, 1994).

Probability of death given illness

The probability of death as the result of choleragenic *V. cholerae* O1 and O139 is dependent on the public health infrastructure of the locality where the case of cholera is acquired. The cornerstone in cholera therapy is rapid oral rehydration. Administration of antibiotics may shorten the duration of diseases (Bennish, 1994). If adequate rehydration is not provided, mortalities range between 20% and 50%. However, in most affected developing countries, mortality rates are less than 5% where oral electrolyte solutions are available (Glass and Black, 1992).

5.4.2. Dose-response model

In this assessment, a dose-response curve was obtained by fitting the approximate Beta-Poisson model

$$\Pr(ill) = 1 - (1 + \frac{dose}{\beta})^{-\alpha}$$

to the data available from several volunteer studies (Cash et al., 1974; Levine et al., 1981, 1988). Firstly, in the study by Cash et al. (1974), volunteers were exposed to a range of doses of the Classical biotype of *V. cholerae* O1 in a food matrix (beef broth). The same organism was given also to human volunteers together with an acid-neutralizing solution. In this current work, a dose-response model was developed using both of these data sets, and resulted in a dose-response curve with higher attack rates at lower doses in volunteers given the organism with an acid-neutralizing solution compared with a food matrix (Figure 2). The study of Cash et al. (1974) was comprehensive as it examined a range of *V. cholerae* doses administered both with a food matrix and with an acid-neutralizing solution. However, as recent studies have shown that the Classical biotype strains are rarely isolated from any part of the world (Sack et al., 2003) this data was not considered to be the most appropriate for developing a dose-response model relevant to current exposure to choleragenic *V. cholerae*.

The studies of Levine et al. (1981, 1988) focused on the El Tor biotype of choleragenic *V. cholerae*. Levine et al. (1981) exposed volunteers to the El Tor biotype of choleragenic *V. cholerae* with a food matrix, acid-neutralizing solution and water. In contrast to the results of Cash et al. (1974), described above, there is evidence provided by Levine et al. (1981) that there is no significant food matrix effect, and that dose-response curves obtained from human volunteer studies where *V. cholerae* doses administered with acid-neutralizing solutions adequately model the consumption of *V. cholerae* with food. In their study, they found that for an El Tor *V. cholerae* given to human volunteers at a dose of 10^6 organisms, a similar response was observed whether the dose was administered with an acid-neutralizing solution, or with a standard meal of fish, rice, custard and skim milk (Levine et al., 1981). The conflicting evidence provided by Levine et al. (1981) compared with Cash et al. (1974) adds to the uncertainty of what the true dose-response curve for *V. cholerae* is. Whether this reflects a difference in the two biotypes or not is not known. However, it is acknowledged that the effect of the food matrix on the dose response when consuming pathogenic vibrios is an important area for future research and represents a critical data gap for this risk assessment. All of these data and the resulting dose-response curves are included in Figure 2. As the study of Levine et al. (1988) looked at a range of doses (10^6, 10^8, 10^{10}), these data were used for the development of a dose-response curve for the El Tor biotype.

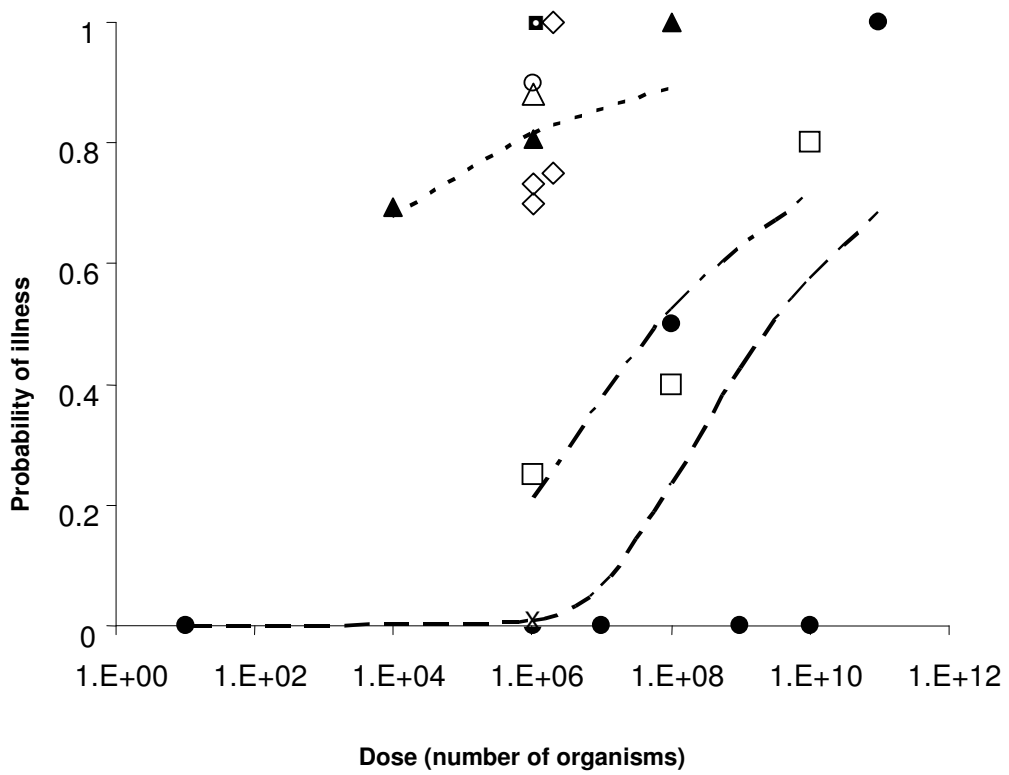

Figure 2. Beta-Poisson dose-response curves for different strains of *V. cholerae*.

KEY: ● = Classical with food matrix (Cash et al., 1974); – – – = fit to Classical with food matrix; □ = El Tor with antacid (Levine et al., 1988); – · – · – · = fit to El Tor with antacid; ▲= Classical Inaba with antacid (Cash et al., 1974); ; - - - - = fit to Classical Inaba with antacid; ◊ = miscellaneous El Tor strains tested; Δ = Classical Ogawa (Cash et al., 1974); ○ = El Tor with bicarbonate (Levine et al., 1981); ◘ = El Tor with food (Levine et al., 1981); X = El Tor with water (Levine et al., 1981).

Figure 2 essentially shows the maximum likelihood fit of the Beta-Poisson model to the available feeding trial data. Analyses of the data were done as described by Teunis et al. (1996) and calculation parameters are indicated in Table 11. The data for both the El Tor and Classical biotypes were analysed. The human volunteer trials conducted by Cash et al. (1974) specifically looked at the effect of *V. cholerae* administered with a buffer and with a meat broth. The column labelled “buffer” in Table 11 indicates whether the dose was administered with a stomach-pH neutralizing buffer, "yes", or "no". A human volunteer study with *V. cholerae* O139 reported similar infectious doses as described for the O1 serotype (Cohen et al., 1999). Note the shift in the dose-response curve in Figure 2 for Classical with food matrix compared with Classical with antacid (Cash et al., 1974). The values derived by Bowers (J. Bowers, CFSAN, personal communication, 2001) are within the confidence intervals of Teunis et al. (1996) and differ slightly because of differences in pooling of severity of illness categories to define cases of illness.

Table 11. Calculation parameters of the dose-response curves for different strains of *V. cholerae.*

Buffer	Alpha (α)	Beta (β)	Deviance	Df	Source
no	0.508	7.52×10^7	1.75	5	Teunis et al., 1996
no	0.1312	1.49×10^7	7.36	5	See note.
yes	0.164	0.149	0.149	1	Teunis et al., 1996
yes	0.119	0.717	0.48	1	See note.
yes	0.113	1.38×10^5	0.526	1	See note.

NOTE: Data on dose-response fitting of *V. cholerae* supplied by J. Bowers, Division of Mathematics, Office of Scientific Analysis and Support, CFSAN.

An assessment of the uncertainty of the dose response was obtained by applying a Bayesian analysis with a non-informative prior for the uncertainty distribution of the parameter values. Specifically, uniform distributions were assumed as priors for the uncertainty of log values of the two parameters of the dose-response function. The prior distribution for $\log_{10}(\alpha)$ was taken to be uniform from -4 to 0 and the prior distribution for $\log_{10}(\beta)$ was taken to be uniform from 2 to 8. These ranges were specified based on the belief that the true dose response is monotonically increasing and low-dose linear. The joint prior distribution was then taken to be the product of these two distributions, and the posterior uncertainty distribution of the parameters was obtained from the data and the prior distribution by Monte Carlo simulation using the WinBUGS software (Spiegelhalter et al., 2003).

The uncertainty distribution of dose-response parameters resulting from the Bayesian analysis are shown in Figure 3. In this figure, the bivariate Bayesian posterior distribution (probability density) of log transformed values is shown based on a kernel smoothing method applied to 1000 Monte Carlo samples obtained from the posterior uncertainty distribution using the WinBUGS software. As evident in the figure, the uncertainty distribution is multi-peaked and falls off rapidly for parameter values that do not lie near the modes (or peaks) of the distribution. In the vicinity of the value of the parameters that maximize the posterior density, the distribution displays a ridge-like feature, indicating a strong correlation between the uncertainty of $\log_{10}(\alpha)$ and $\log_{10}(\beta)$. Alternative sets of parameter values lying along this ridge should be considered equally plausible, given the dose-response data and the prior uncertainty distribution. Also, since the Bayesian analysis was applied using a relatively uninformative prior, best estimates of parameters based on the mode of the posterior distribution are relatively consistent with the maximum likelihood estimate of the dose response. A best estimate obtained from a Bayesian in this fashion is called the maximum *a posteriori* (or MAP) estimate, the Bayesian analogue to the Frequentist maximum likelihood estimate.

For the purpose of the present assessment, the dose-response uncertainty was characterized by the results obtained from the Bayesian procedure. A Monte Carlo sample of 1000 pairs of dose-response parameters was obtained using the WinBUGS software and this sample from the uncertainty distribution was then used for risk characterization. This sample of 1000 pairs of dose-response parameters could be used to characterize the effect of dose-response uncertainty in other subsequent analyses, or a replicate sample could be obtained using the WinBUGS software. The parameters and model for the WinBUGS Monte Carlo simulation are provided in Appendix B.

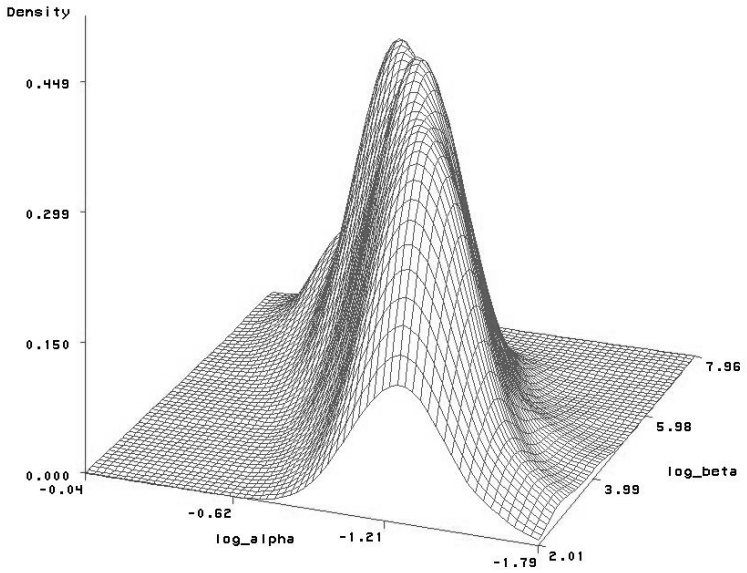

Figure 3. Uncertainty of Beta-Poisson dose-response for *V. cholerae* (Levine et al., 1988). The *a posteriori* uncertainty distribution of the parameters based on a Bayesian analysis of the data with a non-informative prior distribution for log-transformed parameters.

5.4.3. Assumptions regarding the dose-response relationship

As there are gaps in our knowledge base, a number of assumptions were made in developing the dose-response model. The primary assumptions are as follows:

- Healthy volunteer response to oral challenge of *V. cholerae* is representative of the general population.
- The virulence of the pathogen or susceptibility of the host do not vary.
- The Beta-Poisson dose-response model is reasonable for use in characterizing the risk of illness following exposure to *V. cholerae.*
- The dose-response parameters for the El Tor biotype are a suitable surrogate for all of the potential biotypes.

It is acknowledged that these assumptions result in a simplification of the reality as either the lack of appropriate data or the complexity required in developing the dose-response model meant it was not possible to include all factors, such as differences in host susceptibility. As the El Tor biotype is far more commonly isolated in clinical cases of cholera, it was considered appropriate to use the dose-response curve for this biotype in the subsequent quantitative risk-assessment model. Although the dose-response data for the El Tor biotype administered with food is less extensive than that for the Classical biotype, the food matrix effect appeared to be minimal with regard to the El Tor biotype and therefore it was considered appropriate in the

case of this biotype to use data from feeding trials where the *V. cholerae* was administered with an acid-neutralizing solution.

5.4.4. Uncertainty and variability in the estimates

This analysis identifies both uncertainty and variability in the estimates. Since the dose-response estimates are based upon curves fitted to human volunteer data, there is uncertainty as to whether the parameters that give the best fit are the "true" parameters of the dose-response curve. To account for this uncertainty, a Monte Carlo simulation model can be set up to probabilistically select from the group of plausible dose-response parameters generated in this analysis. While it is not modelled in this analysis, it is assumed that there is variability in the virulence of the pathogens and in the susceptibility of the host. Further research is needed to provide data for assessing and modelling variability in the pathogen and in the host. A key uncertainty in this hazard characterization is the effect of the food matrix on the dose-response relationship. Both the experience of the United States of America risk assessment on *V. parahaemolyticus* (FDA, 2001b, 2005) and evidence from the studies of Cash et al. (1974) have indicated that some food matrices may shift the dose-response curve to the right, indicating that a higher dose of the pathogen is required to cause illness. To resolve this question and provide data that will allow scaling factors to be applied to predict the risk for specific pathogens consumed with specific food matrices, it is important that data relevant for dose-response estimations are collected in outbreak investigations. In this assessment, the use of the data from Levine et al. (1988) does not assume a food matrix effect and may be considered to be conservative in that the calculated risk may be higher than the "true" risk.

5.5. Application of the dose-response model

Using human feeding trial data it was possible to develop dose-response curves for both the Classical and El Tor *V. cholerae* biotypes. However, as indicated above a number of assumptions had to be made on generating these dose-response curves. These different curves generated indicated a difference in the dose-response for each of the biotypes which may be an artefact of the manner in which the feeding trials were carried out or may indicate a difference in virulence of the two biotypes. As the El tor is currently the most commonly occurring biotype in clinical cases of cholera the dose-response curve developed for this biotype was used in the estimation of risk in the quantitative risk assessment. The dose-response curve was not directly used for the qualitative risk assessment or the spreadsheet tool. Instead these less complex approaches assumed, based on reports in the literature as indicated in section 5.4.1, that a dose of 10^6 *V. cholerae* cells would cause illness when consumed in food.

6. Risk characterization

Risk characterization is the final step in the risk assessment, and provides a risk estimate that is based on the coupling of the likelihood of consuming an infective dose of the hazard with the severity of the health outcome that the hazard imposes if consumed. Data and outputs from the exposure assessment and hazard characterization steps were used to provide estimates of the likelihood of contracting cholera following consumption of imported warm-water shrimp. As the data available for this risk assessment were limited, it was not possible to undertake a full harvest-to-consumption quantitative risk assessment. Therefore, other approaches were taken to characterize the risk. These included a qualitative approach, the use of a published spreadsheet tool for estimating risk and the development of a quantitative model using data from port-of-entry analysis as the starting point in the exposure pathway considered.

6.1. Qualitative approach to characterizing the risk of contracting cholera from imported warm-water shrimp

Qualitative characterizations of risk are primarily textual descriptions. They are useful when the numerical data necessary to quantify risk are not available, and can help identify where numerical data would be needed to enable quantification of a risk. Qualitative risk assessments follow the same principles as quantitative approaches in terms of logic applied, transparency, need for internal consistency, and data analysis. While there is a perception that qualitative risk assessments are easier than quantitative risk assessments, a number of experts in the field of microbiological risk assessment are of the opinion that this is not necessarily the case if qualitative risk assessment is done using a thorough and scientific approach. For example, without mathematics, many things can be more difficult to explain in a manner that is understood in the same way by all readers. Within the discipline of qualitative risk assessment, risk descriptors are not comparable – rather, they are unique to that risk assessment and the labels used therein cannot be considered equal to labels of the same name used in another qualitative risk assessment. Nevertheless, various forms of qualitative approaches to characterizing risk are used as a basis for taking risk management decisions related to microbiological hazards in foods on almost a daily basis, and it is recognized that such risk assessments have a value in certain situations. The quality and the rigour of method used in qualitative risk assessment will depend on the importance of the decision to be made, and if a very thorough qualitative risk assessment is carried out, it can take just as long as quantitative risk assessment.

The exposure assessment and hazard characterization steps of this risk assessment initially started out as qualitative. Therefore, it was considered that it might be useful to determine if and how such qualitative information could be used to characterize risk. This qualitative approach has been elaborated based on an approach developed by Food Science Australia (FSA, 2000) to describe risk profiles of plant products. In addition, information published by the International Commission on Microbiological Specifications of Foods (ICMSF, 2002) on descriptors for severity of illnesses caused by various pathogens was used as a basis for describing the output of the hazard characterization.

6.1.1. Likelihood of ingesting an infective dose of choleragenic *V. cholerae*

Table 12 provides estimates of the number of servings consumed annually by each consumer in the populations of interest (see also Section 4.2). These estimates indicate that consumption of warm-water shrimp ranges from 6.0 eating occasions in Spain to 0.6 occasions in Germany per year; in the major importing countries, the United States of America and Japan, each consumer has 3.7 and 6.9 servings/annum, respectively. When the proportion of raw:cooked (10:90) consumption of shrimp is included, it becomes clear that, in every country, raw shrimp is consumed (per capita) on less than one occasion per year.

Table 12. Annual servings of warm-water shrimp per capita in the populations of interest.

	Mean annual servings 1995–2000 ($\times 10^7$)	Population ($\times 10^6$)	Servings/caput/annum
USA	110.3	297.0	3.7
Japan	87.9	127.8	6.9
Italy	7.2	57.3	1.3
Spain	24.5	41.1	6.0
Germany	5.1	82.5	0.6
UK	8.9	59.6	1.5
France	17.2	60.4	2.8

NOTE: Population data from FAOSTAT, downloaded March 2005.

The potential for exposure to an infectious dose of choleragenic *V. cholerae* through the consumption of imported warm-water shrimp seems to be very low, based on the data given in Table 6, which indicates only two isolations of choleragenic *V. cholerae* in more than 20 000 port-of-entry analyses of imported warm-water shrimp.

A qualitative approach for the rating of risk has been developed based on premises published by ICMSF (2002) and by FSA (2000). The ICMSF formulated descriptors for severity of illnesses caused by various pathogens. This was used in conjunction with a matrix of factors propounded by FSA (2000) to describe risk profiles of plant products.

Taken together, the present qualitative matrix is based on a number of criteria, as considered below.

Severity

The severity of the identified hazards in terms of their threat to humans was classified according to the ICMSF (2002), with levels of severity defined as follows:

IA. Severe hazard for general population; life threatening, or substantial chronic sequellae, or long duration.

IB. Severe hazard for restricted populations; life threatening, or substantial chronic sequellae, or long duration.

II. Serious hazard; incapacitating but not life threatening; sequellae rare; moderate duration.

III. Moderate, not usually life threatening; no sequellae; normally short duration; symptoms are self-limiting; can be severe discomfort.

The ICMSF categorise *V. cholerae* O1 and O139 as IA but indicates that their involvement in foodborne disease is sporadic and that other factors contribute to the significance of the severity of the threat to health such as the availability of rehydration treatment. The present assessment focuses specifically on *V. cholerae* in warm-water shrimp, processed under GHP

and HACCP, and consumed in importing industrialized countries. Therefore, the population of interest in this assessment is considered to have adequate access to basic medical treatment, which is rehydration with regard to cholera. In addition data provided to WHO by the USA and the United Kingdom indicate 100% sanitation coverage in these countries (WHO, 2000a). It is assumed that this is a reflection of the situation in the industrialised countries that import warm-water shrimp. Given such a scenario it was considered that the severity of threat to human health from choleragenic *V. cholerae* through the consumption of warm-water shrimp could be ascribed to Category II.

Occurrence of illness

This is classified as low, medium or high for the recognized hazards based on involvement of shrimp in causing cholera, as recorded in public health statistics of those countries that keep them. In the present assessment, information presented in Table 8 on cholera statistics for the populations under consideration led to the adoption of a low occurrence of illness.

Growth

An indication is given of whether growth of the pathogen in the product is required to cause disease. In general, microbiological hazards need to grow in the product or be present at high numbers before there is a significant risk of disease. Table 6 presents data that indicate two isolations of choleragenic *V. cholerae* in more than 20 000 port-of-entry analyses of imported warm-water shrimp. This indicates a very low prevalence and level of choleragenic *V. cholerae* in imported warm-water shrimp. As indicated in Section 5, the most commonly reported infectious dose is approximately 10^6 organisms or more (Levine et al., 1981; Tauxe et al., 1994b; Health Canada, 2001; FDA, 2003). While feeding trial data indicate some differences in dose response among the different biotypes and other factors related to the host and food matrix also influence dose response this number is only an approximation. However, given the low prevalence and level of choleragenic *V. cholerae* reported in imported warm-water shrimp it can be assumed that growth of *V. cholerae* in shrimp is required to provide an infective dose.

Production, processing or handling of food

The production, processing or handling of the food may increase, decrease or not affect the hazard. Table 5 presents data that indicated that, during production, *V. cholerae* in raw shrimp undergo inactivation of the order of 5–6 log units, with a cooking step providing a similar quantum of inactivation.

Consumer terminal step

This point considers whether a consumer terminal step, such as cooking, is applied to the product. Cooking by the consumer will, for most biological hazards, reduce the subsequent risk of disease, and Section 4 describes the effect of cooking on *V. cholerae* in shrimp. In the present assessment, it has been assumed that only 10% of shrimp are eaten in the uncooked state and are therefore not subjected to a consumer terminal step.

Epidemiology

Consideration must be given as to whether the hazard-commodity combination has been recorded as a cause of food poisoning. In the case of choleragenic *V. cholerae* in imported warm-water shrimp, despite an exhaustive search of the literature and of country health statistics, no documented case of cholera associated with consumption of warm-water shrimp could be found.

A matrix embracing responses to the above qualitative criteria is presented (Table 13). This indicates that there is very little opportunity for choleragenic *V. cholerae* to survive processing and therefore be present in shrimp that is finally consumed. The very low occurrence of illness and the lack of a documented epidemiological link support this statement. Categorization of this situation in terms of risk was not considered appropriate as any descriptor used might be interpreted in different ways by different people. However, the other information presented should be an adequate basis for decision-making. Such an approach has limited application as it does not quantify the risk; however it can be useful to indicate whether a more detailed risk assessment or quantification of the risk is needed. On the other hand, this level of information may be adequate to facilitate risk management decision making. It should be noted that this approach did not consider the situation where cross-contamination occurs post processing.

Table 13. Summary of the information providing a qualitative description of the risk of acquiring cholera due to the consumption of choleragenic *V. cholerae* in shrimp (after ICMSF, 2002, and FSA, 2000).

Product	Identified hazard	Severity[a]	Occurrence of illness[b]	Growth in product required to cause disease?	Impact of processing and handling on the hazard[c]	Consumer terminal step[d]	Epidemiological link?
Raw shrimp	*V. cholerae*	II	Very low	Yes	Level of hazard reduced during washing (0 – 1 log), icing (2 – 3 logs), freezing (2 – 6 logs)	No	No
Shrimp cooked at the plant and eaten without further heat treatment	*V. cholerae*	II	Very low	Yes	Level of hazard reduced during washing (0 – 1 log), icing (2 – 3 logs), cooking (>6 logs) (optional), freezing (2 – 6 logs)	No	No
Shrimp cooked immediately before consumption	*V. cholerae*	II	Very low	Yes	Level of hazard reduced during washing (0 – 1 log), icing (2 – 3 logs) (optional), freezing (2 – 6 logs), thawing and cooking (>6 logs)	Yes	No

NOTES: (a) Severity level refers to the severity of the identified hazard as classified according to the International Commission of the Microbiological Specifications of Food (ICMSF, 2002). Level II = Serious hazard; incapacitating but not life threatening; sequellae rare; moderate duration.
(b) Very low occurrence of illness can, for the purposes of this risk assessment, be described as an average of less than 1 case per 10 million population per year based on the data that was available over a 6-year period (Table 8). This reflects the situation in all countries considered in this document except Japan, which experienced an average of less than one case per million population.
(c) The total level of inactivation for processing is in the range of a 5–6 log reduction, and the initial contamination level was less than 1 in 25 g.
(d) Cooking of shrimp brings about a 5–6 log reduction in the level of *V. cholerae.*

6.2. Quantitative approaches to characterizing the risk of contracting cholera from imported warm-water shrimp

Qualitative and quantitative risk assessment need not be mutually exclusive. As mentioned earlier, qualitative risk assessment is very useful in an initial phase of risk management and can provide timely information regarding the hazard and the potential of a hazard-commodity combination to cause illness. Such information can assist in deciding on the scope and level of resources to apply to quantitative risk assessment. Moving to quantitative risk assessment means that the data used to estimate the model input parameters is numerical. For example, rather than providing a textual description of the adverse effect of the hazard on the consumer, a numerical description of the dose response can be provided.

In the following sections, two quantitative approaches are described. There are a number of differences between these two approaches. The first approach described uses a simple, spreadsheet-based, food-safety risk-assessment tool, which is available in the peer reviewed literature (Ross and Sumner, 2002). This is a mathematical model with a user-friendly interface that converts qualitative inputs to numerical values and combines them with the quantitative inputs in a series of mathematical and logical steps using standard spreadsheet functions (Ross and Sumner, 2002). These calculations are then used to develop indices of public health risk. It could, perhaps, be therefore considered as a kind of bridging risk assessment between fully qualitative approaches and fully quantitative approaches.

The second approach involved the development of a quantitative risk-assessment model that was developed specifically for this pathogen-commodity combination, based on the available data. As the numerical inputs for a full harvest-to-consumption model were not available, this model was based on a shortened exposure pathway that begins at the port-of-entry in the importing country.

6.2.1. Application of the spreadsheet-based, food-safety risk-assessment tool to characterizing the risk of contracting cholera from imported warm-water shrimp

Version 2[1] of the spreadsheet-based, food-safety risk-assessment tool was used to generate a risk rating and an estimate of predicted illness for a specified scenario or set of conditions. The first version of this tool has been published, and the logic behind the system, as well as its limitations, explained (Ross and Sumner, 2002). The software embodies established principles of food-safety risk-assessment. The tool requires the user to select from qualitative statements or to provide quantitative data, or both, concerning factors that will affect the food safety risk for a specific population from a specific food product and specific hazard during the steps from harvest-to-consumption and uses these inputs to develop indices of public health risk. Further details on how the tool works and guidance for its use are provided in Appendix A.

With its user-friendly interface, such approaches to risk assessment are very appealing. It is an example of how food-safety risk-assessments can be simplified and the benefits of risk assessment made more accessible to risk managers. However, its authors stress that this is not a definitive tool, that there is room for improvement, and that it cannot be considered appropriate for all situations. In addition, any users of the tool should be aware of its limitations. For example, the intent of questions might be misinterpreted; it makes a number of simplifying assumptions; and some of the weighting factors used therein are arbitrarily derived.

[1] The spreadsheet can be downloaded from http://www.foodsafetycentre.com.au/riskranger.htm

Nevertheless, once the user is aware of these and understands how the various questions within the spreadsheet need to be addressed, it can be a useful tool to facilitate decision-making.

Risk ratings were prepared for hazard-product pairings on a scale of 0–100, where zero represents no risk and 100 represents every member of the population eating a meal that contains a lethal dose of the hazard during a single day. The scale is logarithmic and is such that an increment of six in the ranking corresponds approximately to a ten-fold increase in risk.

Version 2 of this tool has been modified from the original published version (Ross and Sumner, 2002) by reducing by a factor of 10 the "weight" given to "Moderate", "Mild" and "Minor" hazard severity classifications (Question 1). This preserves the risk rank scaling (0–100) and its original interpretation, but better reflects the severity of fatal diseases compared with non-life threatening hazards. Question 3 has also been slightly modified to enable better discrimination of serving frequency.

The tool is useful for teaching the principles of risk assessment in relation to food safety, in highlighting factors contributing to food safety risk and in ranking the risk of various pathogen-commodity combinations. As with any such software tool, however, the outputs are only as reliable as the data entered, and users should remain aware of the intended uses and limitations of the software (Ross and Sumner, 2002).

Based on the information presented in the earlier sections, the following data were input to the spreadsheet tool to answer the eleven questions therein. The data inputs to the spreadsheet tool were based on the information available at the time of preparation of this report. Where data were not readily available assumptions were made based on expert opinion. Therefore, the used data inputs used here should be considered as an example of one type of scenario, that of warm-water shrimp which is harvested, processed and distributed under GHPs, HACCP and SSOPs and enters in international trade. However, the user-friendly nature of this tool allows users to answer each of the questions using data representing their specific scenario or situation of concern and receive a risk ranking explicit to that scenario.

Question 1. Hazard Severity?

The spreadsheet tool provides four options as follows:

SEVERE hazard – causes death to most victims

MODERATE hazard – requires medical intervention in most cases

MILD hazard – sometimes requires medical attention

MINOR hazard – patient rarely seeks medical attention

The option: "MODERATE hazard – requires medical intervention in most cases" was chosen as the response to Question 1. As noted by WHO (2004) medical intervention in cases of cholera is straightforward, basically rehydration, and when applied appropriately the case fatality rate will be less than 1%. Without medical intervention the case-fatality rate may reach 30 – 50%.

Question 2. How susceptible is the population of interest?

The spreadsheet tool provides four options as follows:

GENERAL – all members of the population

SLIGHT – e.g. infants, aged

VERY – e.g. neonates, very young, diabetes, cancer sufferers, alcoholics, etc.

EXTREME – e.g. AIDS, transplant recipients, etc.

For the current assessment: "GENERAL – all members of the population" was selected.

Questions 3 and 4: Frequency of consumption and proportion consuming?

The spreadsheet tool provides five options for frequency of consumption as follows:

DAILY

WEEKLY

MONTHLY

A FEW TIMES PER YEAR

OTHER ("number of days between a 100-g serving")

In addition, it provides the following four options for proportion of population consuming the product:

ALL (100%)

MOST (75%)

SOME (25%)

VERY FEW (5%)

Data on servings in each population of interest (Table 12) were modified to fit the options available in the spreadsheet tool, using the following process:

- Of total servings, 10% were considered to be consumed raw and 90% consumed cooked.
- The total number of annual servings per capita in each country was calculated for raw and for cooked consumption.
- In each country, an estimate was made of the frequency of consumption (Question 3) and the proportion consuming imported warm-water shrimp (Question 4). To comply with the format required by the spreadsheet-based tool, the data presented in Table 12 had to be converted from specific numbers to the percentage of consumers that eat this product (all (100%); most (75%); some (25%); very few (5%)) in a specified period (daily; weekly; monthly; a few times per year; other). According to the data presented in Table 12, in the United States of America, for example, 3.7 servings of imported warm-water shrimp are consumed per head of population annually. As it is being assumed that 90% is eaten cooked and 10% is eaten raw, this equates to 0.3 servings of raw shrimp per person per year and 3.4 servings of cooked shrimp per person per year (Table 14). Taking the example of raw shrimp, of which 0.3 servings are estimated to be consumed per person per year, it means that much of the population never eat raw shrimp. Rather than saying 100% of the population eat 0.3 servings per year, this can be expressed as 10% of the population eating 3 servings of raw shrimp per year. The closest option available in the spreadsheet-based tool in terms of number of consumers is "very few" (5%), so this was selected. The number of servings, i.e. 3, was expressed as "a few times per year". Similarly for cooked shrimp, 3.4 servings per person per year was expressed as 25% of the population eating 13.6 servings per year, i.e. a serving per month (Table 14).

Table 14. Annual servings of warm-water shrimp per capita consumed raw and cooked in the populations of interest .

	Warm-water shrimp consumed raw		Warm-water shrimp consumed cooked	
	Total per capita per annum	Selected consumption and frequency alternative for spreadsheet tool	Total per capita per annum	Selected consumption and frequency alternative for spreadsheet tool
USA	0.3	Very few (5%) eat few times a year	3.4	Some (25%) eat monthly
Japan	0.7	Some (25%) eat a few times a year	6.2	Most (75%) eat monthly
Italy	0.1	Very few (5%) eat a few times a year	1.2	Some (25%) eat a few times a year
Spain	0.6	Some (25%) eat a few times a year	5.4	Most (75%) eat monthly
Germany	0.1	Very few (5%) eat a few times a year	0.5	Very few (5%) eat monthly
UK	0.1	Very few (5%) eat a few times a year	1.4	Some (25%) eat a few times a year
France	0.3	Very few (5%) eat a few times a year	2.5	Some (25%) eat monthly

Question 5. Size of consuming population?

In the spreadsheet tool, two options are provided for answering this question: select from a menu of countries and territories, or input the population size of interest. For the purposes of this assessment, the populations in each selected country listed in Table 12, rounded to the nearest million, were used.

Question 6. Probability of contamination of raw product per serving?

For this question, the spreadsheet tool provides six options as follows:

RARE (1 in a 1000)

INFREQUENT (1%)

SOMETIMES (10%)

COMMON (50%)

ALL (100%)

OTHER ("enter a percentage value between 0 (none) and 100 (all)")

As this risk assessment focuses on choleragenic *V. cholerae* O1 and O139 this was an important consideration when reviewing the available data. Dalsgaard et al. (1995a) found that *V. cholerae* O1 was present in 2% (2/107) of water, sediment and shrimp samples collected from a major shrimp culture area in South-east Asia, though testing of isolates indicated absence of the *ctx* genes in both (Dalsgaard et al., 1995b). Data from India showed the presence of *V. cholerae* O1 in 0.2% of raw shrimp (Ministry of Agriculture, India, personal communication, 2001). However, the choleragenic status of these shrimp-associated strains is unknown. Data submitted to FAO/WHO from Argentina (M. Costagliola, personal communication, 2001) indicate the absence of *V. cholerae* O1 and O139 in 400 shrimp and 15 water samples examined. As two of the above studies did not detect choleragenic *V. cholerae* in shrimp samples and the third did not determine the choleragenic status of the *V. cholerae* detected it was assumed that the probability of contamination of incoming shrimp with choleragenic *V. cholerae* was 0.1%.

While not stated, implicit at Question 6 is the need to estimate a concentration of *V. cholerae* in incoming shrimp. In the absence of any information, a premise used by other researchers has been used, which, at its simplest, states that, if the prevalence is low, the concentration is also likely to be low.

Prevalence and concentration of pathogens in foods are often considered to be related properties, particularly at very low concentrations. The observed prevalence will depend on the sample size and the extent of contamination of the batch. If the batch is contaminated at a level of >1 cfu/g, there is high probability that, in each 25-g sample, the pathogen of concern would be detected. If, however, the sample size were only 1 g, some samples would not contain cells of the pathogen. If the contamination level were 1 per 100 g, only one in four 25-g samples would be expected to "test positive", and it is then more usual to describe this concentration as "25% prevalence".

In fact, the distribution of bacteria in a sample is likely to follow a Poisson distribution. In that case, if the mean concentration is X per gram, and there are Y grams per sample, the count per sample is Poisson distributed with mean X*Y. More importantly, the probability of a positive result for a sample of Y grams is then: 1 -exp(-X*Y). Thus, for large amounts of product, prevalence and concentration are related and the estimate of the prevalence depends on the level of contamination and the sample size.

Similarly, products that permit the growth of pathogens may exhibit a low prevalence of contamination at the point of production and a higher prevalence at the point of consumption. This is not necessarily due to re-contamination but may arise because the product was initially contaminated at a very low level. Subsequent growth in the product increases the probability of detection of that contamination.

Accordingly, in the present study, a concentration of 10 cfu/g was used (the limit of detection), equivalent to 2 750 cfu per serving. This concentration is used to estimate an answer to Question 10 (increase to infectious dose).

Question 7. Effect of processing?

For this question, the spreadsheet tool provides seven options as follows:

The process RELIABLY ELIMINATES hazards

The process USUALLY (99% of cases) ELIMINATES hazards

The process SLIGHTLY (50% of cases) REDUCES hazards

The process has NO EFFECT on the hazards

The process INCREASES (10×) the hazards

The process GREATLY INCREASES (1000×) the hazards

OTHER ("enter a value that indicates the extent of risk increase")

Table 5 illustrates the effect of processing on inactivation of *V. cholerae* for which a >5 log inactivation is documented during washing, icing and freezing. For Question 7, the alternative: "other" is selected and a 4-log inactivation inserted. Based on the information presented in Table 5, this is a conservative estimation.

If shrimp are cooked during processing, the lethality of the temperature-time regimes used in the industry are greatly in excess of 6 log units (see Section 4.2.2), which leads to selection that the hazard is "reliably eliminated" at this stage.

Question 8. Re-contamination?

The spreadsheet tool provides four options as follows:

NO

YES – minor (1% frequency)

YES – major (50% frequency)

OTHER ("enter a percentage value between 0 (none) and 100 (all)")

Re-contamination post-cooking (from water, ice or workers) of shrimp processed under GHPs, HACCP, GMPs and SSOPs is considered to be minimal. However, there was no actual data available to indicate the frequency of recontamination. Therefore, an assumption had to be made with regard to this frequency. In undertaking this work no reports of choleragenic *V. cholerae* in warm-water shrimp in international trade were identified. As noted earlier even during the Peruvian cholera epidemic in of the early 1990s it could be isolated from only 1 out of 1011 samples of seafood at factory level that were destined for export and choleragenic *V. cholerae* O1 was not isolated during increased sampling and analyses of imported shrimp from South American countries during that period (DePaola et al., 1993). This data would appear to indicate that recontamination is not an important factor and where good knowledge of a shrimp processing facility is available it would be reasonable to select "No" recontamination as the response to this question. As this work sought to provide a more global perspective on risk and recognize that system failures may occassionally occur, 0.0001% was selected as the frequency of recontamination. If reliable data are available on recontamination then they can be easily substituted as the answer to this question following the guidance provided in Appendix A.

Question 9. Effectiveness of post-processing controls?

For this question, the spreadsheet tool provides five options, as follows:

WELL CONTROLLED – reliable, effective systems in place (no increase in pathogen)

CONTROLLED – mostly reliable systems in place (3-fold increase)

NOT CONTROLLED – no systems, untrained staff (10-fold increase)

GROSS ABUSE OCCURS – (e.g. 1000-fold increase)

NOT RELEVANT – level of risk agent does not change

The cold chain in international trade (frozen and chilled) is well established and *V. cholerae* has a minimum temperature for growth of 10°C (see Section 4.2.2). However, loss of control can occur which may potentially give rise to problems. For the purposes of this risk assessment the option "controlled" was selected. In terms of applying this spreadsheet tool, if specific information are available on the effectiveness of post process controls then another option may be selected.

Question 10. Increase in the initial contamination level (Question 6) that would cause infection or intoxication to the average consumer?

For this question, the spreadsheet tool provides five options, as follows:

NONE

SLIGHT (10-fold increase)

MODERATE (100-fold increase)

SIGNIFICANT (10 000-fold increase)

OTHER ("what is the increase needed to reach an infective dose")

Based on the data presented in the literature (Levine et al., 1981; Tauxe et al., 1994b; Health Canada, 2001; FDA, 2003) and the information presented in Figure 2 a dose of 1 million (10^6) *V. cholerae* was assumed to be the infective dose. In a serving of 275 g, such an ID_{50} is equivalent to a concentration of approximately 3 600 cfu/g of shrimp at the point of consumption. To answer Question 10, it is necessary to divide the level at Question 6 by the level for an infective dose. In this case:

Infective dose = 10^6

Level from Question 6 = 10^3 (275-g serving with a concentration of 10 cfu/g = 2 750 g/serving)

The difference is approximately 10^3, and this value is used at Question 10.

Question 11. Effect of preparation before eating?

The spreadsheet tool provides five options as follows:

Meal preparation RELIABLY ELIMINATES hazards

Meal preparation USUALLY ELIMINATES (99%) hazards

Meal preparation SLIGHTLY REDUCES (50%) hazards

Meal preparation has NO EFFECT on hazards

OTHER ("enter a value that indicates the extent of risk increase")

Where shrimp are cooked, it is likely that this will result in complete elimination of *V cholerae*. The organism is not heat tolerant and the location of the site of microbiological concern – the carapace – means that heat treatment will be immediate. Thus, any form of cooking (steaming, boiling or barbecuing) will result in complete elimination. Where shrimp are eaten raw, there is no effect on the hazard.

Risk ratings and predicted illnesses

Table 15 presents risk ratings and predicted illnesses based on consumption of raw warm-water shrimp in the seven countries for which risk was assessed. Risk ratings varied between 25 (Japan and Spain) and 21 (all others), the difference reflecting higher consumption per capita in the former countries. In assessments of seafood hazard-product pairings in Australia, Sumner and Ross (2002) found that the spreadsheet tool ratings <30 were not equated with any reports of illness.

Predicted illnesses were very low, with 1 to 2 cases of cholera caused by consumption of warm-water shrimp in a decade predicted for Japan, the United States of America, and Spain. For the other countries considered, 3 to 4 cases per century were predicted i.e. approximately 1 case every 25 years. Differences in predicted illness reflect population differences between countries, plus per capita consumption rates.

In terms of consumption of cooked shrimp, it is considered that the hazard is reliably eliminated during cooking either at the plant level (Question 7: Reliably eliminates) or during meal preparation (Question 11: Reliably eliminates). Thus for such a scenario the spreadsheet tool will not predict any cases of cholera.

It should be emphasized that the these predictions follow from the inputs to the spreadsheet-based tool described above. For example, as highlighted earlier in this section there are no data

available on the frequency of recontamination of warm-water shrimp post-processing and given the rarity at which choleragenic *V. cholerae* has been isolated from processed warm-water shrimp it could also have been assumed that recontamination never takes place. If this single input was changed the spreadsheet tool predicts ten times less cases of illness in the population. It is a straightforward process to substitute these new inputs into the spreadsheet and further details on how to do this are provided in Appendix A. Therefore, should different or additional inputs become available, or a user has data specific to his/her particular situation or country, for example data may be available from HACCP programs that indicate that the processing steps undertaken always achieve a 6 log reduction, then this tool can be readily used to determine the impact of such data.

Table 15. Estimation of risk associated with consumption of raw warm-water shrimp in selected countries.

Risk criteria	Japan	Spain	USA	Italy	UK	France	Germany
Dose and severity							
Hazard severity	Moderate						
Susceptibility	General – all population						
Probability of exposure							
Frequency of consumption	Few times a year						
Proportion consuming	Some (25%)	Some (25%)	Very few (5%)	Very few (5%)	Very few (5%)	Very few (5%)	Very few (5%)
Size of population	128 million	41 million	297 million	57 million	60 million	60 million	83million
Probability of contamination							
Probability of raw product contaminated	0.1% (10 cfu/g)						
Effect of processing	4-log inactivation						
Possibility of recontamination	0.0001%						
Post-process control	Controlled						
Increase needed to reach infective dose	1000×						
Further cooking before heating	No effect						
Predicted illnesses per year in selected population	0.2	0.1	0.1	0.03	0.03	0.03	0.04
Risk ranking (0–100)	25	25	21	21	21	21	21

6.2.2 Application of a quantitative model to assess the risk of contracting cholera from imported warm-water shrimp

As mentioned earlier, the lack of quantitative data meant it was not possible to undertake a fully quantitative harvest-to-consumption risk assessment for this pathogen-commodity combination. Therefore, a quantitative model was developed based on quantitative data available in importing countries – one that essentially covers the steps from the port-of-entry in the importing country to the point of consumption within that country (Figure 4).

The main data inputs for the exposure assessment component of this model were the volumes of shrimp imported into selected countries (Table 7), the results of port-of-entry analysis for choleragenic *V. cholerae* in imported warm-water shrimp (Table 6) and the populations of the selected importing countries (Table 12). The Beta-Poisson dose-response model developed using data for the El Tor biotype of *V. cholerae* (Levine et al., 1988) and described in Section 5 was used. In undertaking this risk assessment, it was assumed that 10% of imported warm-water shrimp was consumed in the raw state. It was also assumed that, in the case of shrimp consumed after cooking, that the hazard had been eliminated either during meal preparation or during cooking at the processing plant. It is accepted that, in the latter case, recontamination was possible, but the likelihood of this event occurring was judged to be minimal (see Section 4.2.2 for supporting justification).

Overview of modelling approach

The low prevalence of choleragenic *V. cholerae* O1 in exported warm-water shrimp (around 0.1% (Table 6)) makes quantitative modelling of both variability and uncertainty via Monte Carlo simulation problematic in that a very large proportion of shrimp portion servings are expected to have no contamination and therefore no risk. Thus, if a simulation of exposure and risk per serving on the level of individual servings were undertaken, a very large number of servings would need to be simulated in order to obtain an adequate (Monte Carlo) approximation to the risk associated with a small (but non-zero) probability of exposure. While techniques are available to overcome this they was not feasible in the development of this model. Instead, mean exposure and mean risk were evaluated analytically within a Monte Carlo simulation of the effect of uncertainty. The uncertainties considered here were: (a) the prevalence of *V. cholerae* O1 in imported warm-water shrimp samples; and (b) the dose-response of choleragenic *V. cholerae* O1. The uncertainty associated with prevalence observations was modelled as a Beta distribution with parameters inferred based on the Bayesian *a posteriori* analysis assuming no other prior information. The uncertainty of the dose-response estimates was characterized using a form of Bayesian analysis (see Section 5).

The available import testing data for warm-water shrimp, namely data for Japan (1995–2000), the United States of America (2000) and Denmark (1995), were selected (Table 6). Because of similarities in methodologies for isolation of *V. cholerae*, it was agreed that pooling available testing data from these countries was reasonable. Based on the observed prevalence of choleragenic *V. cholerae* O1 and O139 in 25-g shrimp samples tested at the point of import, a corresponding density of *V. cholerae* O1 and O139 was estimated by assuming a Poisson distribution of *V. cholerae* in shrimp. This distribution is rational, given the low frequency of detection, although other (e.g. mixed) distributions might be consistent with the observed prevalence. The prevalence of *V. cholerae* in shrimp was assumed to be homogeneous for multiple national sources. Data on the prevalence of positive samples were not pooled across

different years of data, in order to analyse how risk changed over time. Furthermore, it was assumed that, due to changes in the shrimp industry (e.g. shrimp handling, processing, and implementation of GHP and HACCP), the density of *V. cholerae* O1 in tested shrimp products may vary from one year to the next.

Given the (Beta) uncertainty distribution for the year-specific prevalence of positive detection, a corresponding uncertainty distribution for the mean density corresponding to a given prevalence of detection was taken to be

$$\lambda = -\log(1 - p)$$

where λ is the mean of the Poisson distribution and p is prevalence. This relation between the density (λ) and the prevalence (p) is derived from the assumption of the Poisson distribution, whereby the probability of observing a positive sample is:

$$1 - \exp(-\lambda)$$

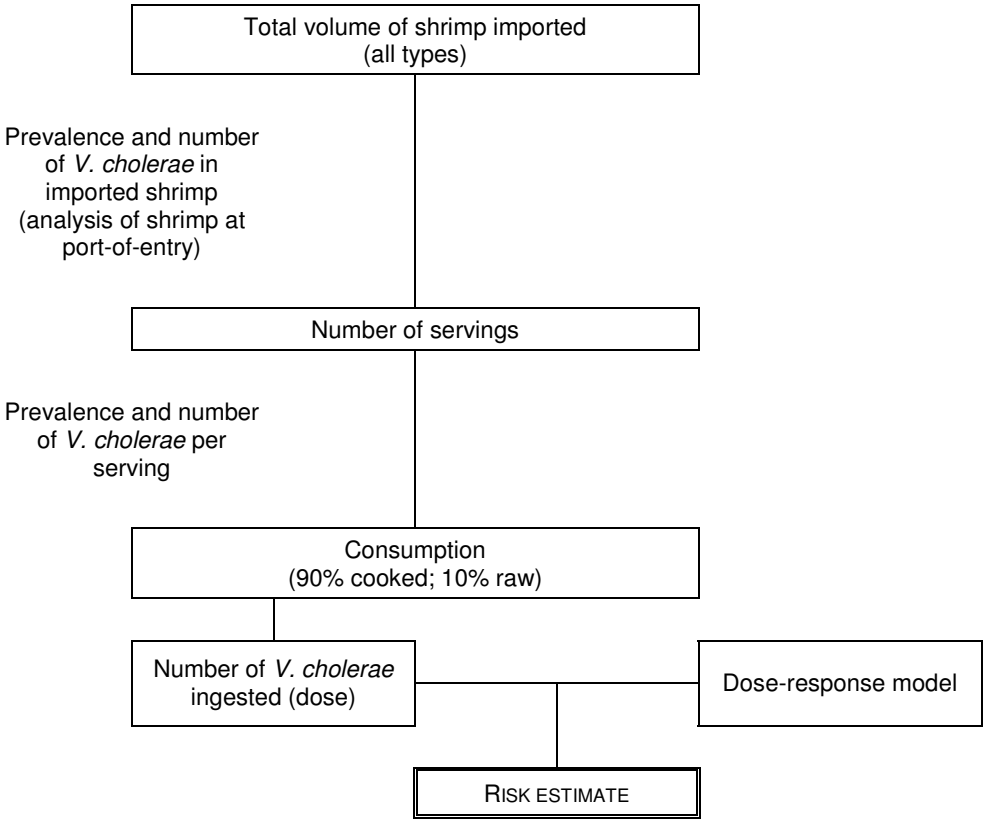

Figure 4. Import-to-consumption pathway used as the basis for the development of a quantitative risk-assessment model to estimate the risk of acquiring cholera from imported warm-water shrimp.

Outputs

Using this model, the annual risk per serving from imported warm-water shrimp was calculated. In addition, the risk per country per year was calculated based on the risk per serving per year and the number of servings consumed in a particular country in a particular year.

The estimated risk of becoming ill with cholera following consumption of imported warm-water shrimp on a yearly basis for each of the seven countries for which data were available is presented in Table 16. For Japan, for example, the 50% or median risk was between 0.21 and 0.93 over the 6-year period examined. This is equivalent to 1 to 5 cases every 5 years. As there have been no reports of cholera associated with warm-water shrimps, this estimate may be high, reflecting the conservative assumptions of the quantitative model. However, it would also difficult for a disease surveillance system to pick up such a disease incidence level. Even though cholera is a notifiable disease it is still estimated that only 50% of all cholera cases are reported (Mead et al., 1999; Adak, Long and O'Brien, 2002). Risk is linked to the amount of shrimp consumed: the higher the level of consumption (Table 12), the greater the risk. Thus, the differences in the risk between countries are based solely on the differences in the numbers of servings.

The risk per country per year was calculated based on the risk per serving per year and the number of servings consumed in a particular country in a particular year. The risk per serving, i.e. the likelihood or probability (95%; mean; 5%) of a case of cholera occurring as a result of consuming a serving of imported warm-water shrimp was estimated as shown in Table 17. These estimates indicate that the median risk of getting cholera from a serving of imported warm-water shrimp was between 2.14×10^{-9} and 9.68×10^{-9}. In 2000, for example, it was estimated that 3 out of every 1000 million servings would result in a case of cholera illness. At the upper, 95%, limit, the chance of a cholera case occurring was 4.95×10^{-7}, that is around 5 out of every 10 million servings would result in a case of cholera illness, and at the lower, 5%, limit the probability was 2.37×10^{-11}, that is 2 out of every 100 000 million servings would result in a case of cholera illness. It is important to remember that these estimates are likelihoods. Even though the likelihoods are very low, it is still possible that someone somewhere could get cholera from the consumption of imported warm-water shrimp, particularly if the number of servings of shrimp are very high.

As the risk was estimated over a period of six years, consideration was given to whether risk changed over time. In 1996, the estimated risk was lower in all countries than in 1995. In 1995, two shrimp samples tested positive for *V. cholerae* and as a result the estimated risk for that year was higher. However, from 1996 to 2000, the risk appeared to be very slowly increasing. This is likely to be a relic of the fact that the number of shrimp samples tested also decreased over time and therefore, with a smaller number of tests, there is less information and certainty on the absence of *V. cholerae* O1 and O139 in the shrimp. However, if surveillance of shrimp harvesting and processing practices were somehow incorporated into the model, then it is likely that a reverse trend of decreasing risk would be seen. Such a trend would reflect greater observed adherence to safe shrimp harvesting and processing practices.

Table 16. Estimated risk, on a yearly basis, of becoming ill with cholera following consumption of imported warm-water shrimp

Country importing warm-water shrimp		Estimated number of cholera illness cases occurring in the year					
		1995	1996	1997	1998	1999	2000
USA	95% risk	108.01	30.06	41.93	48.19	56.63	62.07
	median risk	0.96	0.21	0.28	0.33	0.39	0.42
	5% risk	0.010	0.001	0.002	0.002	0.003	0.003
Japan	95% risk	105.43	29.60	35.04	33.34	38.45	42.31
	median risk	0.93	0.20	0.24	0.23	0.26	0.28
	5% risk	0.010	0.001	0.002	0.002	0.002	0.002
Italy	95% risk	7.63	2.37	2.47	3.24	3.28	3.77
	median risk	0.068	0.016	0.017	0.022	0.023	0.025
	5% risk	0.0007	0.0001	0.0001	0.0002	0.0002	0.0002
Spain	95% risk	24.31	7.06	8.85	11.37	11.85	13.65
	median risk	0.22	0.048	0.060	0.076	0.081	0.10
	5% risk	0.0023	0.0003	0.0004	0.0005	0.0006	0.0007
Germany	95% risk	5.28	1.37	2.04	2.23	2.36	2.81
	median risk	0.047	0.009	0.014	0.015	0.016	0.019
	5% risk	0.0005	0.0001	0.0001	0.0001	0.0001	0.0001
UK	95% risk	8.94	2.53	3.14	3.76	4.35	5.40
	median risk	0.079	0.017	0.021	0.026	0.030	0.036
	5% risk	0.0008	0.0001	0.0001	0.0002	0.0002	0.0003
France	95% risk	17.06	5.28	6.43	7.88	8.64	8.37
	median risk	0.151	0.036	0.043	0.054	0.059	0.056
	5% risk	0.0016	0.0003	0.0003	0.0004	0.0004	0.0004

Table 17. Annual risk per serving from imported warm-water shrimp.

	Risk per serving					
	1995	1996	1997	1998	1999	2000
95% risk	1.09E-06	3.13E-07	3.92E-07	4.20E-07	4.69E-07	4.95E-07
median risk	9.68E-09	2.14E-09	2.64E-09	2.86E-09	3.22E-09	3.32E-09
5% risk	1.02E-10	1.51E-11	1.83E-11	1.96E-11	2.27E-11	2.37E-11

7. Gaps in data

Lack of quantitative data from harvest to consumption

There are limited data on the densities of choleragenic *V. cholerae* O1 and O139 in the environment, at harvest, during processing and after preparation of warm-water shrimp, and it has rarely been detected in any shrimp. The quantitative approach for modelling exposure in this risk assessment largely circumvented these data limitations by simply using test data to estimate *V. cholerae* levels at consumption. With such a low frequency of detection of choleragenic *V. cholerae* O1 and O139 (~1 in 10 000 samples) in imported warm-water shrimp, the estimation that the level was 1 per 25 g in these positive samples is statistically supportable, but the density would need to be adjusted upward with increasing frequency of detection (i.e. consumption in developing countries). It is also worth noting that the methodology that has been used in most studies to date may not be optimal for isolating choleragenic *V. cholerae* and newer methodology using an enrichment step at 42°C rather than 35°C might be more appropriate when testing for choleragenic *V. cholerae*. The application of such methodology may lead to more quantitative data being available in the future.

The lack of quantitative data also extended to the testing data for importing countries in which risk was modelled. While such data were not available from many of the selected countries, it was considered that the warm-water shrimp imported by most countries came from the same sources, and therefore the test data from Denmark, Japan and the United States of America was considered applicable to all countries. However, the availability of more country specific data would mean that the risk assessment approaches described could be adapted to that country.

Data on consumption

There was no specific data on the consumption of shrimp in the seven countries considered in this risk assessment. In this work, volumes of shrimp imported were used to estimate amount of shrimp consumed.

Data on levels of faecal cross-contamination during handling of shrimp

Shrimp have occasionally tested positive for choleragenic *V. cholerae* O1 and O139, though from ecological data it appears that these organisms are not part of the natural biota of shrimp. Therefore, contamination during handling is the most likely source of choleragenic *V. cholerae* O1 and O139, but data to support the origin and level of contamination is lacking.

Data to clarify the dose response when the El Tor biotype is ingested

There is an apparent discrepancy between the studies of Cash et al. (1974) and Levine et al. (1981) regarding the effect of the food matrix on the dose response of choleragenic *V. cholerae* O1 and O139. More data are necessary to know whether this difference is due to actual difference between the Classical and El Tor biotypes, or the effect of different food matrices on choleragenic *V. cholerae* effective dose. The effect of the food matrix on the effective dose of pathogens is an important data gap for this risk assessment and other foodborne pathogen risk assessments for which human volunteer feeding studies are available.

8. Key findings and conclusions

There is a high degree of alignment between the three approaches taken, particularly in the steps of exposure assessment and hazard characterization. The differences lie primarily in how these approaches attempt to use the data from the previous steps to characterize risk. The qualitative approach noted in particular the low occurrence of cholera in importing countries, the lack of a documented epidemiological link between cholera cases and imported shrimp, the apparently low occurrence of choleragenic *V. cholerae* in shrimp, and very limited opportunity for *V. cholerae* in raw shrimp to survive the processing and preparation of shrimp for consumption. In the case of shrimp that were cooked either at the processing plant or during meal preparation, the hazard was considered to have been eliminated. However, little consideration was given to cross-contamination, and if such an approach were to be applied, particularly in cholera-endemic countries, this would be an important issue to address.

In many situations, the qualitative approach should provide adequate information for a risk manager to conclude that warm-water shrimp processed under strict GHPs and HACCP does not pose a problem in terms of a source of cholera. However, qualitative approaches can also be used as a basis on which to build more detailed quantitative approaches that incorporate more quantitative data. Risk assessments function by amassing the best data available at any given time, and, as a matter of conformance with Codex Principles and Guidelines for the Conduct of Microbiological Risk Assessment (CAC, 1999), both uncertainties and data gaps should be documented in a risk assessment report. However, the difficulty in quantifying the output, and the associated uncertainties, of qualitative approaches may limit their application.

While every attempt has been made to transparently document the qualitative approach, it was also decided to use this opportunity to build upon the qualitative work and develop more quantitative approaches to assessing the risk. Accordingly, two quantitative approaches were elaborated: (i) using a published spreadsheet-based, risk-assessment tool; and (ii) a fully quantitative model based on the available quantitative data. The spreadsheet tool provided a more quantitative estimation of the risk, and provided a description of how such a tool can be used to estimate the risk associated with choleragenic *V. cholerae,* thus serving as guidance on the application of this tool. This spreadsheet-based tool is publicly available to download from the Internet and therefore it is possible to input data for a specific scenario and obtain an associated risk estimate. There are limitations to this tool, as described in Ross and Sumner (2002), which should be taken into account in any application of the tool. The guidance provided in Appendix A should also be followed when using this tool. Based on the data used as inputs to the spreadsheet-based tool, 1 to 2 cases of cholera caused by consumption of warm-water shrimp in a decade was predicted for Japan, the United States of America, and Spain and approximately 1 case every 25 years in the other countries considered.

A quantitative risk assessment was undertaken in order to further reduce the uncertainty in estimating a low risk. However, the data limitations associated with this approach were such that the pathway considered had to be shortened to where it began at the point of entry of imported shrimp into a country. Consequently, no specific consideration was given to harvesting, processing or distribution. Rather, sufficient information on the level of contamination of warm-water shrimp resulting from all these steps was considered to be

contained within the outcome of testing data at the point of entry of the importing country. Apart from taking into account the fact that 90% of shrimp were assumed to be consumed cooked and 10% consumed raw, this risk assessment did not give any consideration to growth-allowing deviations from cold chain temperatures during transport, storage or distribution within the importing country, or storage or preparation at the food service or home level. Therefore, the exposure model can only be considered as a very simplistic representation of shrimp handling. As many of its assumptions are conservative from a public health perspective, it has merit in expressing risk; however, it is also useful to demonstrate that there are many different ways in which a risk assessment can be developed. In comparing these approaches, one must recognize the limitations as well as the benefits associated with each.

This quantitative risk assessment provided estimates that the likelihood of contracting cholera from imported warm-water shrimp lies between 0.009 and 0.9 cases per country per year. These estimates can also be considered very low, although there are some differences between these and the outcomes of the semi-quantitative risk assessment. Consideration has been given as to the different factors that contribute to the spreadsheet-based tool and the quantitative risk model, as they result in somewhat different estimates, although both can be considered to be in agreement in terms of predicting low levels of risk. Some of the differences are considered below.

The pathways considered by both of these risk assessments are different. While the spreadsheet-based, risk-assessment tool considers the harvest-to-consumption pathway, the quantitative risk assessment considers a much shorter pathway. Consequently, the inputs to both risk assessments are different, particularly in terms of the exposure assessment. The spreadsheet-based tool has much more emphasis on the effect of various processing steps that would bring about a reduction in the number of *V. cholerae* in the shrimp. In contrast, the full quantitative risk assessment is considering what is essentially a ready-to-eat product with no specific consideration given to steps that might bring about a reduction in organism numbers in the shrimp.

There are also differences in the way in which dose response is considered in both approaches. The spreadsheet-based tool basically takes a point estimate approach and assumes the infectious dose to be one million organisms. It uses an Exponential dose-response model, which has a steeper slope than the Beta-Poisson approach used in the quantitative model. The spreadsheet-based tool is a generic tool which can be applied to a range of pathogen-commodity combinations. The quantitative approach was developed specifically for this product- pathogen pair and it was agreed that the Beta-Poisson model was the most appropriate description of the available feeding trial data. If the Exponential model were to be fitted to the data set of Levine et al. (1988), then a low-dose slope for the dose-response curve would be approximately 100-fold less than that of the Beta-Poisson low-dose slope. Thus, if such a fitted Exponential dose-response model were used to predict numbers of cases in the quantitative risk assessment, then the risk would decrease by roughly 100-fold. This difference would appear to explain most of the differences in the output of the two approaches. In spite of this difference, it still remains that both approaches predict a small likelihood of contracting cholera from consuming warm-water shrimp moving in international trade.

A continuing challenge in performing pathogen-commodity microbiological risk assessments is the limited data available with which to model exposure and dose response. In such cases, it

is difficult to get reliable estimates of the standard deviation around the data, making the comparison of different approaches even more problematic.

The development of this report has provided an opportunity to bring together and examine a large body of research on cholera and shrimp production. Besides providing a risk estimate, there were a number of other worthwhile findings from the analysis of data used in this risk assessment. While *V. cholerae* is widely distributed in the environment, only strains producing cholera toxin belonging to serotypes O1 and O139 are causative agents of cholera, and such strains are rarely isolated from shrimp from aquatic environments. This is important in terms of sampling and analysis for choleragenic *V. cholerae*, and highlights the importance of testing any strains isolated for their choleragenic potential. There are a number of steps in shrimp processing that bring about substantial reductions in *V. cholerae* and therefore contribute to the low levels of *V. cholerae* hitherto found in imported shrimp.

Finally, predictions of low risk by each of the approaches taken is supported by absence of epidemiological evidence that imported warm-water shrimp have ever been incriminated in a cholera outbreak in any developed nation in the world.

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Appendix A

GUIDANCE ON THE USE OF THE SPREADSHEET-BASED TOOL

Introduction

The spreadsheet is intended as a simple food safety risk calculation tool to help determine relative risks from various product-hazard combinations it. In particular, it is meant to make the techniques of food safety risk assessment more accessible to non-expert users, and to users with limited resources, both as a decision-aid and an educational tool.

The spreadsheet incorporates all factors that affect the risk from a hazard in a particular commodity including:

- Severity of the hazard and susceptibility of the population of interest
- Likelihood of a disease-causing dose of the hazard being present in a meal
- Number of meals consumed by a population of interest in a given period of time

Using the Tool

The Tool is a Microsoft ® Excel spreadsheet that includes a series of "list boxes" from which you choose answers to respond to eleven questions by using your computer mouse or by entering specific numerical values. A mathematical model then converts the descriptive answers to numerical values and applies mathematical and logical rules to convert the numbers into an estimate of relative risk. The mathematics and logical rules are detailed in Ross and Sumner (2002)[1].

In general, answering the questions should be straightforward but, because of the logic used in the tool, some additional guidance is needed to answer some questions appropriately. This following provides additional advice on how to answer the questions.

Answering the questions

Question 1: Hazard severity

Question 1 assesses the severity of the hazard being considered. It offers four choices. Table A1 presents examples of hazards that would fit those descriptions. One may disagree with these descriptions of hazard severity. If you believe a description is wrong for the specific country or region under consideration, select the hazard severity that you consider appropriate. Remember, when documenting your risk assessment you will have to explain your selection of the hazard severity.

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[1] Ross, T. and Sumner, J. (2002). A simple, spreadsheet-based, food safety risk assessment tool. *International Journal of Food Microbiology*, 77, 39-53.

Table A1. Associating hazards with descriptions at Question 1

Description	Consequences of the hazard	Hazard Example
Severe	Death in most cases	Tetrodotoxin, Botulinum toxin
Moderate	Most cases require medical treatment	*Listeria monocytogenes, Vibrio vulnificus, Vibrio cholerae*, Enterohaemorrhagic *E. coli* (EHEC)
Mild	Sometimes medical treatment is needed	*Vibrio parahaemolyticus*, Hepatitis A, Norwalk-like viruses, Histamine, Ciguatera, Algal biotoxins, *Salmonella*
Minor	Medical treatment rarely required	*Staphylococcus aureus*, *Clostridium perfringens*

Question 2: Susceptibility of the population in which you are interested

The tool asks you to describe the vulnerability of your 'population of interest' to the hazard being considered. Four choices of susceptibility are given, and these are briefly explained below:

- **General** – includes everyone in the country or region of interest.
- **Susceptible** - small children (1-5 years old) and people over 65 years old are more susceptible to many types of foodborne hazard than the "general" population. In the tool, the magnitude of that increased susceptibility is assumed to be five-fold.
- **Very susceptible** - includes newborn babies, children under one year of age and people with conditions such as diabetes, cancer, liver damage etc. which predispose them to infectious diseases. They are rated in the tool as 30-times more susceptible than the general population.
- **Extremely susceptible** – includes people with HIV-AIDS, for example, or those recovering from transplant surgery that have very weakened or suppressed immune systems. These people are considered to be 200-times more likely to succumb to hazards than the general population

The relative susceptibility "weightings" (5x, 30x and 200x) were based on the relative susceptibility of each population sub-group to systemic infection by *Listeria monocytogenes*. (Details of the reasons for these weightings, based on epidemiological data, are given in Ross and Sumner, 2002). Consequently, the selected weighting may lead to unexpected results if applied to hazards to which all people are more or less equally susceptible, for example, *S. aureus* enterotoxin. You can, if appropriate or necessary to better reflect the susceptibility of the sub-population of interest, alter the relative susceptibility values. Instructions for recoding of the weightings in the tool are given at the end of this Appendix.

When you select a sub-population, it means that you want to assess the risk to that sub-population only, among the general population. Once you have made this selection here, the spreadsheet automatically makes adjustments so that you do not have to consider the size of this sub-population when answering Question 5, nor further consider the susceptibility of the selected sub-population at Question10.

Question 3: Frequency of consumption

This question, and Question 4, helps you estimate the exposure of the average consumer, among the selected population, to the product (whether it contains the hazard or not). You can select

from the choices provided in the list-box or include some other value. To do this, select "Other" in the list, and then type a value for the average number of days between consumption of a serving of the product by an average consumer in your population of interest.

Question 4: Proportion of population consuming the product

This question works together with Question 4 to estimate exposure of the selected population to the number of servings of the food. It explicitly recognises in the calculations that not all people in the population of interest will consume the product that contains the hazard. You can select from four choices: **all** (100%), **most** (75%), **some** (25%) and **very few** (5%) of the population who eat the product on some regular basis.

You can answer Questions 3 and 4 using either of two methods:

- Using consumer survey data which gives you a very good idea of consumption patterns
- Calculating the amount consumed from product harvest (or slaughter or catch) statistics and then dividing by the population you think eats that product. Obviously you need to make an assumption here and to record within the risk assessment the assumptions you have made.

Where the actual consumption data do not readily match the choices given for combinations of consumption frequencies and proportions of the population consuming the product, another combination that leads to the same overall consumption, or exposure, within the selected population can be used. This will not greatly affect the risk estimates. An example of this approach is given in Section 6.2.1 of this risk assessment document. Remember, however, that if this strategy is used, it should be documented and the rationale explained (as shown in Section 6.2.1).

Question 5: Size of consuming population

The spreadsheet has several country populations programmed into Question 5. If you want to assess the risk in another country, or restrict your risk assessment to a specific region, select "Other" in the list box, and type the population of that country/region in the "Other" box[2].

Note that if you selected a specific sub-population in Question 2, the spreadsheet automatically estimates the number of people in the selected country or region that are in that category so that you do not need to do so when answering this question, i.e. this question relates to the total population in the country or region of interest. However, because the spreadsheet was developed in Australia, the proportions of consumers in the various susceptibility categories are appropriate to that country. While those proportion are probably appropriate to other countries with similar lifestyles, you may need to recalibrate the coding for Question 2 for countries in which population susceptibility may differ, e.g. countries with a high prevalence of AIDS. Instructions for recoding of the weightings in the tool are given at the end of this Appendix.

Question 6: Probability that a serving of raw product is contaminated

To answer this question you need specific knowledge about the likelihood of contamination of the product with the hazard. If you have data from a properly designed survey you can insert the exact level by selecting "Other" in the list box, then typing the value as a **percentage** in the box

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[2] If you want to make the list box specific to your nation, click the tab for CODINGS, go to Item: "10:Size of Consuming Population" in columns D and E and insert your own populations in rows 26-39.

below. Alternatively, if you do not have an accurate idea of the proportion contaminated, you can select the most appropriate description from the list box.

Question 7: Effect of processing

To answer this question you need to know about the process and how it affects the hazard, as well as the likely level of the hazard that is initially present. For example:

- If you're considering viruses in oysters that are intended to be consumed raw, their numbers are not affected by processes such as shucking or storage, so you select "No effect on the hazard". The same selection would be made for ciguatera in reef fish, because the level of toxin is not affected by the process.
- If you're considering *Vibrio* spp. in cooked shrimp, the cooking process is sufficient to kill all realistic levels of the bacteria so select "Reliably eliminates hazard".
- If you were considering the risk from *L. monocytogenes* on refrigerated, vacuum-packed processed meats in which growth is possible, you can select either "Increases the hazard" or "Greatly increases the hazard" depending on the reliability of the temperature control and typical time between production and consumption.
- If you freeze fish or crustacea, a proportion of the vegetative bacteria such as *Vibrio* spp. are likely to die - perhaps as many as 50% - and the option "SLIGHTLY (50% of cases) REDUCES" can be selected.

Question 8: Potential for recontamination after processing

Recontamination is particularly important for those products contaminated with microbial hazards that receive a heat treatment during the process. Such products have low bacterial levels and introduced microbial contaminants will be able to grow with little competition. Examples of where recontamination is important include:

- Cooked, peeled shrimp recontaminated with *Staphylococcus aureus* from the hands or noses of food handlers.
- Hot smoked salmon recontaminated during slicing and packing with *L. monocytogenes* from the environment.
- Canned seafood recontaminated through a leaking seam with *Clostridium botulinum* from seawater used in cooling.

To answer Question 8 you really need data generated from surveys and this can be typed in the "Other" box. If you don't have data on recontamination you can make an assumption based on observation or on comparison with similar processes which have been surveyed in countries with conditions similar to your own. For example, if you observe operators peeling shrimp with their bare hands you might expect that up to 50% of the product will be (re-)contaminated, because 30-50% of food handlers carry *S. aureus* on their hands and nose.

Question 9: How effective is post-processing control?

To answer this question you need to know how the product is handled during storage, distribution and retailing and also how the hazard responds to those conditions. Here are some examples:

- Bacterial pathogens in frozen seafood cannot increase, and may even die, so select "Well controlled" from the list box.
- The population of viruses in oysters will remain static during storage so select "Not relevant". The same applies to ciguatera in reef fish – processing doesn't affect toxin level.
- In smoked seafoods stored at 4-5°C, *L. monocytogenes* will be able to multiply. If the shelf-life is long (4-6 weeks) growth can be significant so select "Large Potential for Growth".
- In chilled, ready-to-eat seafoods such as cooked shrimp, stored at 4-5°C, *L. monocytogenes* will grow, but because the shelf-life is only short, increase in growth will not be great so select "Controlled".

Question 10: What increase in the initial contamination level (Question 6) would cause infection or intoxication to the average consumer?

To answer this question you need to know something about the amount of the hazard that would be required to cause illness and the level expected to be present at the end of processing. Table A2 presents some data on the number of organisms it takes to make a healthy person ill. These data are presented for guidance purposes only and it is well recognized that not every microbiologist and other experts in this area will agree with them. As recognized in this risk assessment as well as others the infective dose will according to characteristics of the host and the strain of microorganism as well as the vehicle of transmission. The numbers are given for a 100g serving, so the count/g of food is 100x lower. It is with great trepidation that these numbers are presented here because not every microbiologist will agree with them. If you believe a number is wrong and have good evidence, it is strongly advised to use the most up to date available data to answer Question 10.

Table A2. Examples of levels of pathogenic bacteria which are likely to cause illness in healthy people and may be used to answer Question 10 in the absence of more specific data on the infectious dose.

Organism	Infective dose in a 100g serving
Salmonella	10,000,000
Listeria	10,000,000,000
Viruses (Hepatitis A, Norwalk)	10-100
Staphylococcus aureus	100,000,000

Note that the answer to Questions 6 is used to estimate the answer to Question 10 which, in effect, is an estimate of the dose of the hazard or pathogen required to make 50% of average consumers ill ("ID50"). This information is used to gauge how much increase in the level of the pathogen could be tolerated before it led to a high probability of infection upon ingestion. The model then works out the effect of processing (Question 7) and recontamination (Question 8) and subsequent distribution/storage/display (Question 9) to estimate how close the contamination level is, prior to cooking, to the "ID50".

In the absence of any information, a premise used by other researchers can been used. At its simplest, this premise considers that, if the prevalence is low, the concentration is also likely to be low. The observed prevalence will depend on the sample size and the extent of contamination of the batch. If the batch is contaminated at a level of >1 cfu/g, there is high probability that, in each 25-g sample, the pathogen of concern would be detected. If, however, the sample size were only 1 g, some samples would not contain cells of the pathogen. If the contamination level were 1 per 100 g, we would expect only one in four 25-g samples to "test positive", and it is then more usual to describe this concentration as "25% prevalence".

In fact, the distribution of bacteria in a sample is likely to follow a Poisson distribution. In that case, if the mean concentration is X per gram, and there are Y grams per sample, the count per sample is Poisson distributed with mean X*Y. More importantly, the probability of a positive result for a sample of Y grams is then: 1 -exp(-X*Y). Thus, for large amounts of product, prevalence and concentration are related and the estimate of the prevalence depends on the level of contamination and the sample size.

Note also, that it is not necessary to consider the answer to Question 9 when answering Question 10.

The software compares the difference between:

- the initial contamination level (Questions ,6, 7 and 8) and
- the level that is believed to be required to cause illness, e.g. Table 2,

to the increased expected (answer to Question 9) and estimates the dose in the product just before the product is eaten or cooked.

To calculate the answer for Question 10 divide the number below in (2) into the number in (1).

1. The number of each organism required to cause illness in *normal, healthy people.*
2. The level of contamination in the raw product (response to Questions 6)

For example, if you're considering *L. monocytogenes* in smoked seafood, you probably won't know the contamination level after processing and recontamination. The literature tells us the contamination level will probably not exceed 10/g, so if we consume 100g there are 1000 cells in our serving just after processing. If we assume that we need around 10,000,000,000 to make us ill, the increase to infective dose is 10,000,000-fold and we can enter that in the "Other" box at Question 10.

Question 11: Effect of meal preparation

This question considers the form of cooking and preparation for cooking. Here are some examples of how you can answer Question 11:

- Cooked shrimp kept chilled until consumption – select "No effect".
- For histamine in tuna, staphylococcal toxin in cooked crustaceans, ciguatera in reef fish or algal biotoxins in bivalves select "No effect" because the toxins are heat-stable.
- For raw seafood contaminated with vegetative pathogenic bacteria that will be thoroughly cooked select "Reliably eliminates".

Spreadsheet outputs: risk estimates

The spreadsheet combines the factors in Questions 1-11 using simple mathematics and some logical rules to generate three estimates of risk:

- Risk Ranking – a score between 0-100
- Probability of illness per day in the selected (sub-)population
- Predicted annual illnesses in the population you selected

Full details of the logic and equations leading to the risk estimates are given in Ross and Sumner (2002).

Risk ranking

The risk ranking value is a scaled logarithmically between 0 and 100. The former is equated to a probability of food-borne illness of less than, or equal to, one case per 10 billion people (greater than current global population) per 100 years. At the upper limit (Risk Ranking=100), every member of the population eats a meal that contains a lethal dose of the hazard every day. A risk ranking change of 6 corresponds to 10-fold difference in the absolute risk. Thus, an increase in risk ranking from 36 to 48 means that the risk increased 100-times.

Predicted annual illness

The spreadsheet estimates the total number of illnesses in the population you select at Question 5.

Probability of illness per day in target population

The spreadsheet targets the proportion of the population which you select at Question 2. Risk ranking remains the same, irrespective of whether you're considering the general population, or a highly susceptible sub-population. But the probability of illness increases in the target population. This output tells you which consumers experience the greatest burden of disease.

Recoding of assumptions in the Spreadsheet Tool

If you click on the "CODING#2" tab at the bottom left side of spreadsheet you will switch to the codings for each question. Do not change any values in Column F.

Relative Susceptibility

To recalibrate the proportions in the various susceptibility categories, got to cells A8 to A11, which describe the susceptibility categories. In the adjacent cells (B8 to B11) are the susceptibilities to the hazard of those sub-populations compared to the susceptibility of the general population (which is always given the weight 1). To change the relative susceptibility weightings, enter appropriate values in cells B9 to B11.

Proportions Susceptible

To recalibrate the proportions in the various susceptibility categories, go to cells D46 to D49, which describe the susceptibility categories. In the adjacent cells (E46 to E49) are the proportions of the general population that are considered to fall into each of these categories in Australia. If these proportions are different for different hazards or in different nations or communities, enter appropriate values in cells D46 to D49.

Country (or Region) Populations

To change the countries or regions available in the list in Question 5, go to cell D25. In cells D26 to D39 are listed countries, with corresponding populations (at 2006) in cells E25 to E39. Any of these countries and corresponding populations can be altered. Do not alter D40, D41, E40 or E41

Appendix B

PARAMETERS AND MODEL FOR THE WINBUGS MONTE CARLO SIMULATION

The WinBUGS program used to fit the dose-response data is:

```
model
{
 for(i in 1:3)
{
 p[i] <- 1-pow(1+d[i]/pow(10,logb),-pow(10,loga))
 d[i] <- pow(10,logd[i])
 x[i] ~ dbin(p[i], n[i])
}
 loga ~ dunif(-4,0)
 logb ~ dunif(2,8)
 a <- pow(10,loga)
 b <- pow(10,logb)
}
 list(logd = c(6, 8, 10),
 x = c(1, 2, 4),
 n = c(4, 5, 5))
```

Briefly, the 4 lines within the curly brackets {...} following “model” at the very top define what we are trying to fit to the data (the Beta-Poisson model), where p[i] is the risk at the i-th dose group (d[i] is the dose; x[i] is the number of observed illnesses; n[i] is the number exposed). The next two lines (loga ~ dunif(-4,0); logb ~ dunif(2,8) are the assumed “priors”). The 3 lines at the bottom are the data (3 dose groups for Levine et al., 1988).

Appendix C

EUROPEAN UNION RAPID ALERT CONCERNING *V. CHOLERAE* IN SHRIMPS

As this risk assessment was in the final stages of completion, the European Union initiated a Rapid Alert citing "detection of *V. cholerae* in raw and cooked frozen black tiger shrimps (*Penaeus monodon*) from Bangladesh, notified by Sweden". Alert notifications are sent when the food presenting the risk is on the market and when immediate action is required. Alerts are triggered by the Member State that detects the problem and has initiated the relevant measures, such as withdrawal or recall.

The Alert notification impinged directly on Exposure Assessment data presented in the present risk assessment, and enquiries were made of the Swedish authorities for further data. These data were made available with commendable promptness and are as follows[1]:

Product:	*Penaeus monodon* (Black tiger shrimp), raw, frozen
Country of origin:	Bangladesh
Means of transport:	Ship
Date of sampling:	10/05/2005
No. of samples:	Aggregate sample of 3 samples; random sampling
No. of positives:	The aggregate sample was positive for *Vibrio cholerae*
Methods:	NMKL 156-2
	PCR: Non-toxigenic *Vibrio cholerae*
	Agglutination: non O-1, non O-139

The analysis indicates presence of non-toxigenic *V. cholerae* (non O-1, non O-139) in raw shrimp (not in cooked as originally noted in the Alert). Both the Rapid Alert and the subsequent laboratory analysis are seen as both timely and important for the present risk assessment.

- Firstly, authorities need to react to a primary screen that indicates the presence of pathogens in cooked products that might be eaten without further consumption, and a Rapid Alert system is appropriate.

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[1] Appreciation is expressed to Ms Ulrika Evans Cederlund, National Food Administration, Sweden, for her assistance in providing these data in a timely manner.

- Secondly, more intensive laboratory analysis, as in the present case, that indicates that non-toxigenic *V. cholerae* is detected in composite samples in raw product only, places the Alert in an entirely different risk context.

FAO/WHO MICROBIOLOGICAL RISK ASSESSMENT SERIES

1 Risk assessments of *Salmonella* in eggs and broiler chickens: Interpretative Summary, 2002

2 Risk assessments of *Salmonella* in eggs and broiler chickens, 2002

3 Hazard characterization for pathogens in food and water: Guidelines, 2003

4 Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: Interpretative Summary, 2004

5 Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: Technical Report, 2004

6 *Enterobacter sakazakii* and microorganisms in powdered infant formula: Meeting Report, 2004

7 Exposure assessment of microbiological hazards in food: Guidelines, 2005

8 Risk assessment of *Vibrio vulnificus* in raw oysters: Interpretative Summary and Technical Report, 2005

9 Risk assessment of choleragenic *Vibrio cholerae* 01 and 0139 in warm-water shrimp in international trade: Interpretative Summary and Technical Report, 2005

10 *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting Report, 2006