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Risk assessment of *Vibrio vulnificus* in raw oysters

INTERPRETATIVE SUMMARY AND TECHNICAL REPORT

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AND TECHNICAL REPORT

WORLD HEALTH ORGANIZATION
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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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 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 we live in. 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 world 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 to 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 Department of Food Safety, Zoonoses and Foodborne Disease, 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.

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.

Ezzeddine Boutrif

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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 and/or international trade. 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 caused 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 Japan, New Zealand, Australia, Canada 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 *V. vulnificus* in raw oysters. When this work began, a draft risk assessment model had been developed in the United States of America to assess the public health risk associated with *V. parahaemolyticus* in raw oysters. The above-mentioned FAO/WHO *V. parahaemolyticus* risk assessment in raw oysters looked at how to adapt the model developed in the USA to the situation in other countries. The purpose of this risk assessment was to investigate further the utility of the United States draft *V. parahaemolyticus* model, which was published in 2001[1], together with the additional work undertaken by FAO and WHO on the risk assessment of *V. parahaemolyticus*, and determine if it could be adapted to a different pathogen, *V. vulnificus.* The current risk assessment was developed in the same raw oyster vehicle and also, for reasons of data availability, for the same geographic region, thereby representing a very similar exposure scenario. This risk assessment was also undertaken because *V. vulnificus* illness has one of the highest mortality rates of any foodborne disease and has emerged as a food safety issue in a number of countries and regions including Europe, Japan, New Zealand, Republic of Korea and the USA.

[1] The final version of the United Stated Food and Drug Administrations Quantitative Risk Assessment on the Public Health Impact of Pathogenic *Vibrio parahaemolyticus* In Raw Oysters was released on 20th July 2005. This current work does not take into account any changes that were made to the original draft model (2001) and which were included in this just published version of the risk assessment.

ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CDC	Centers for Disease Control (USA)
COVISS	Cholera and other Vibrios Surveillance System
FAO	Food and Agriculture Organization of the United Nations
FAO/WHO-VPRA	Joint FAO/WHO Risk Assessment of *V. parahaemolyticus* in Raw Oysters
FDA	Food and Drug Administration (USA)
FDA-VPRA	U.S. FDA Draft Risk Assessment on the Public Health Impacts of *V. parahaemolyticus* in Raw Molluscan Shellfish
g	gram
GCSL	FDA Gulf Coast Seafood Laboratory, Dauphin Island (USA)
ICP	Interim Control Plan for *Vibrio parahaemolyticus*
ISSC	Interstate Shellfish Sanitation Conference (USA)
Kg	Kilogram
LOD	Limit of detection
MLE	Maximum Likelihood Estimate
MPN	Most Probable Number
NBDC	National Buoy Data Center (USA)
NERRS	National Estuarine Research Reserve System (USA)
NOAA	National Oceanic and Atmospheric Administration (USA)
NSSP	National Shellfish Sanitation Program (USA)
PCR	Polymerase Chain Reaction
ppt	parts per thousand
RAPD	Random Amplification of Polymorphic DNA
USDA	United States Department of Agriculture
VBNC	Viable but non-culturable
VPRA	*Vibrio parahaemolyticus* Risk Assessment
Vv	*Vibrio vulnificus*
WHO	World Health Organization

INTERPRETATIVE SUMMARY

INTRODUCTION

This risk assessment was undertaken as one of five pathogen-commodity combinations addressed in the FAO/WHO risk assessment work on *Vibrio* spp. in seafood. Within that framework *Vibrio vulnificus* was identified as one of the three *Vibrio* spp. responsible for most cases of human illness caused by vibrios, where seafood was the vehicle of transmission. There have been at least two previous risk assessments for *Vibrio vulnificus*. McCoubrey (1996) reported on the risk of *V. vulnificus* infection following consumption of raw commercially harvested North Island oysters from New Zealand in 1996. The European Commission's Scientific Committee on Veterinary Measures relating to Public Health has prepared a document on *V. vulnificus* and *Vibrio parahaemolyticus* in raw and undercooked seafood (Scientific Committee on Veterinary Measures relating to Public Health, 2001). However, neither of these risk assessments was quantitative.

In considering approaches to undertake a risk assessment on this pathogen in seafood, with limited available resources, it was decided to extend the *V. parahaemolyticus* models described in the United States FDA "Draft Risk Assessment on the Public Health Impacts of *V. parahaemolyticus* in Raw Molluscan Shellfish" ("FDA-VPRA") (FDA, 2001) and the Joint FAO/WHO Risk Assessment of *V. parahaemolyticus* in raw oysters ("FAO/WHO-VPRA") (FAO/WHO, in press) to *V. vulnificus*. The general approach and many of the parameters used in the current *V. vulnificus* risk assessment are the same as those used in the draft FDA-VPRA and FAO/WHO-VPRA.

Objective and scope

The first objective of this risk assessment was to determine the usefulness of adapting the FDA-VPRA and FAO/WHO-VPRA models to assess the risk from *V. vulnificus* septicaemia associated with the consumption of raw oysters. Secondly, the risk assessment aimed to identify the most appropriate data, as well as gaps in the available dataset, for modelling purposes. In addition to estimating the risk of *V. vulnificus* septicaemia associated with the consumption of raw oysters, the risk assessment model was also developed with the objective of evaluating targeted mitigation levels aimed at reducing the risk of *V. vulnificus* septicaemia. For reasons of data availability, the risk assessment was limited to consideration of primary septicaemia cases associated with consumption of raw oysters from the Gulf Coast of the United States of America (USA).

HAZARD IDENTIFICATION

V. vulnificus naturally inhabits warm estuarine environments and can infect humans via wound exposure or seafood consumption. These infections are rare and generally limited to individuals with pre-existing chronic illnesses or the immunocompromised. However, *V. vulnificus* can invade through the intestinal barrier into the bloodstream causing primary septicaemia. As a result, it has the highest case/fatality rate (approx. 50%) among foodborne pathogens. While *V. vulnificus* has been found in a variety of seafood's worldwide and illnesses have been reported in a number of countries, its epidemiology, ecology and distribution in seafood's have been most extensively investigated in the USA. Each year, 30 to 40 oyster-associated primary septicaemia cases are reported in the USA and nearly all of

these are associated with the consumption of raw oysters harvested from the Gulf Coast in the south of that country.

EXPOSURE ASSESSMENT

A schematic diagram of the *V. vulnificus* risk assessment model is shown in Figure 1. Modelling exposure to *V. vulnificus* followed the same approach and used many of the same assumptions as used for the FAO/WHO-VPRA and the FDA-VPRA. The model inputs, data sources and assumptions are summarized in Table 1.

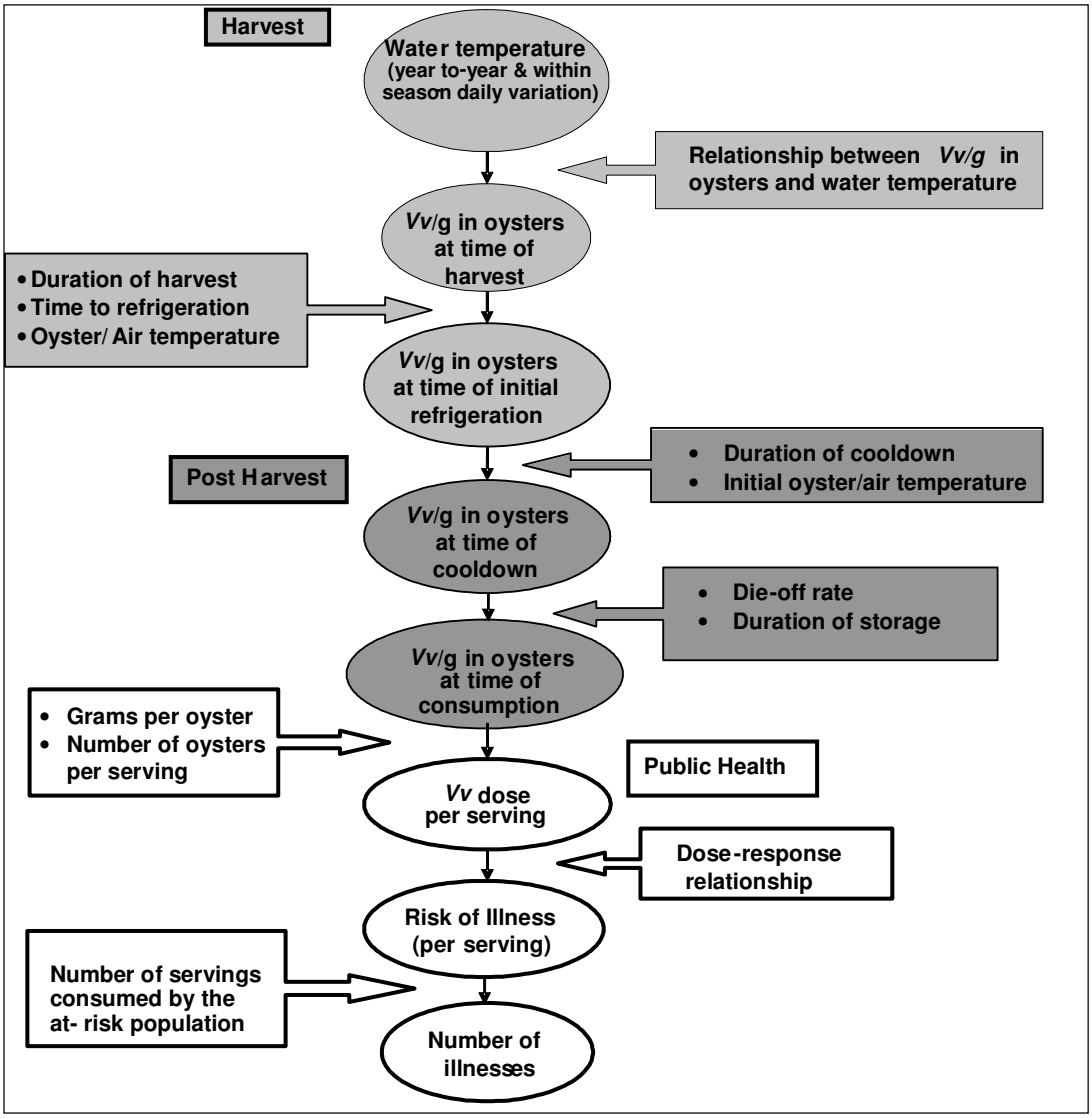

Figure 1. *V. vulnificus* (*Vv*) conceptual risk assessment model showing integration of all modules.

Table 1. Model inputs, data sources and assumptions for the proposed *V. vulnificus* risk assessment

Model Inputs	Data Source	Assumptions
Water temperature	FAO/WHO-VPRA/FDA-VPRA; NOAA buoy data (NOAA, 1999); and NERRS (NERRS, 2001)	Buoy and other fixed site data are representative of growing areas.
Total *V. vulnificus* numbers at harvest	Weekly oyster samples from 4 Gulf States 1994–1995 (Motes et al., 1998; Tamplin, 1994)	Data relevant for other years.
Pathogenic *V. vulnificus* numbers at harvest	Weekly oyster samples from 4 Gulf States 1994–1995 (Motes et al., 1998; Tamplin, 1994)	All *V. vulnificus* strains are equally virulent. Data from Jackson, Murphree and Tamplin (1997) and two recent studies (Nilsson et al., 2003; DePaola et al., 2003) suggest opposing view on this subject, but neither addresses seasonal or regional differences in virulence.
Air temperature	FAO/WHO-VPRA/FDA-VPRA; NOAA buoy data (NOAA, 1999)	Temperature of oyster meat equilibrates rapidly to that of air.
Time harvest vessel in water	FAO/WHO-VPRA/FDA-VPRA; Dealer survey (Cook, 1997b)	Harvest practices have not changed since 1996.
Time to first refrigeration	FAO/WHO-VPRA/FDA-VPRA; Dealer survey (Cook, 1997b)	Oysters are harvested at a constant rate throughout the harvest period.
V. vulnificus growth rate	Natural populations in oysters at ambient temperature (Cook, 1997a). Data lacking at lower temperature	*V. vulnificus* grows at similar temperature-specific rates in Gulf oysters from April to October.
V. vulnificus/g at first refrigeration	Dealer survey of Gulf oysters 1995–1996 (Cook, 1997b)	Dealer practices in 1996 are typical of current practices.
Cool down time	FAO/WHO-VPRA/FDA-VPRA; no data	Rectangular (uniform) distribution between 1 and 10 h.
V. vulnificus/g at cool down	Dealer survey of Gulf oysters 1995–1996 (Cook, 1997b)	Dealer practices in 1996 are typical of current practices.
V. vulnificus survival	Natural populations in oysters stored at 3°C for 14-17 d (Cook et al., 2002)	*V. vulnificus* die off at 3°C similar to that at other temperatures between 0-13°C.
Pathogenic *V. vulnificus*/g at consumption	Retail study of USA oysters 1998–1999 (Cook et al., 2002)	Data are relevant for other years. (The autumn of 1998 was extremely warm and *V. vulnificus* levels were considerably higher than levels predicted for typical autumn temperatures).
Percentage of population susceptible	Prevalence of liver disease, immune disorder, etc., in the USA (Klontz, 1997; Desenclos et al., 1991; Shapiro et al., 1998; Hlady, 1997)	All predisposing conditions have been identified and risk of infection is homogeneous with respect to these conditions. There is no risk of illness (septicaemia) to individuals without the identified disease conditions.
Percentage of oysters consumed raw	50% oysters consumed raw; FAO/WHO-VPRA	Susceptible individuals consume raw oysters at the same rate as the total population.
No. of oysters per serving	FAO/WHO-VPRA/FDA-VPRA	Consumption behaviour has not changed appreciably in recent years.
Weight per oyster	Retail study of USA oysters 1998–1999 (Cook et al., 2002)	None.
Risk of illness	Relationship between monthly exposure and illness within defined (Gulf States) population (Cook et al., 2002; M. Glatzer, personal communication, 2001; NMFS, 1998)	Consumption of raw oysters among the susceptible population does not vary from month to month. (If the percentage of raw oysters consumed was greater in cooler months, the effect of the assumption would be to over-predict the risk at lower *V. vulnificus* levels). Reporting of *V. vulnificus* septicaemia cases related to oyster consumption does not vary from month to month.
Number of illnesses	Risk of illness (from above) multiplied by number of servings	Same as above for risk of illness.

The assumption that all strains are equally virulent is based primarily on animal models (DePaola et al., 2003) that may not be valid for humans, as is suggested by other studies. Those of Jackson, Murphree and Tamplin (1997) and Nilsson et al. (2003) indicate that only a few strains of the diverse populations of *V. vulnificus* found in oysters are associated with human disease. However, there is no definitive test available to measure virulence and no data available to determine their seasonal and regional distribution or their ability to grow and survive under typical industry practices and proposed interventions. Thus, in the absence of definitive information to the contrary, it was assumed that all strains are equally virulent.

Like *V. parahaemolyticus*, *V. vulnificus* numbers at harvest are determined primarily by water temperature and salinity. Other factors may also contribute to *V. vulnificus* numbers but only temperature and salinity have been quantified (Motes et al., 1998; Tamplin, 1994). The numbers of *V. vulnificus* at consumption are influenced by the ambient air temperatures at harvest; the time from harvest until the oysters are placed under refrigeration; the time it takes the oysters to cool once under refrigeration; and the length of refrigeration time until consumption. The growth model used in the present assessment is the three-phase linear growth model advocated for microbial risk assessment by Buchanan, Whiting and Damert (1997).

Two data sets were identified for estimating total *V. vulnificus* numbers relative to the water temperature at harvest. Analyses of these data sets separately and combined pointed to substantial differences, which indicated that it was not appropriate to combine them (simplistically) into a single (pooled) data set. The sensitivity of the exposure assessment to the apparent differences between these data sets was evaluated by conducting alternative assessments based on each data set separately as well as combined (or pooled). The results of these analyses were then compared with available data on the density of *V. vulnificus* in oysters at retail (Cook et al., 2002) for validation. This comparative validation approach suggested that the estimates based on the Motes et al. (1998) dataset were a more appropriate basis for model predictions.

Overall, the model simulation results suggest that *V. vulnificus* numbers increase post-harvest an average of 0.90 $\log_{10}$ MPN per gram during the summer harvest season and decrease an average of 0.2 $\log_{10}$ MPN per gram during the winter harvest season. Mean densities of 57 000 and 80 organisms per gram were obtained for the summer and winter harvest seasons, respectively. Given an average serving size of 196 g of oyster meat (Cook et al., 2002; A. DePaola, personal communication, 2002), these mean densities correspond to average ingested doses of 1.1×10^7 and 1.6×10^4 respectively.

HAZARD CHARACTERIZATION

The virulence factors associated with *V. vulnificus* include a capsule, cytolysin, protease/elastase and phospholipase, but these are found in nearly all clinical and environmental strains (Strom and Paranjpye, 2000). Virulence appears to be multifaceted and is not well understood, thus all strains are considered virulent.

Foodborne *V. vulnificus* infection is clearly associated with underlying medical conditions (Strom and Paranjpye, 2000). Liver disease is a prominent risk factor for *V. vulnificus* infection, including cirrhosis due to alcohol consumption. Additional risk factors include diabetes, gastrointestinal disorders (surgery, ulcers), haematological conditions, and immunodeficiency due to underlying conditions such as cancer and treatment of chronic

conditions with immunosuppressive agents (e.g. arthritis). *V. vulnificus* may pose a small risk to otherwise "healthy" individuals since a small fraction of cases (<5%) are reported to occur in individuals without any identifiable risk factor. The prevalence of predisposing conditions among the adult population (>18 years of age) in the USA has been estimated in a 1997 memorandum to the FDA Office of Seafood Director (Klontz, 1997). These numbers suggest that approximately 7% of the adult population in the USA is susceptible to infection. Given the uncertainty in prevalence of liver disease (including hepatitis), this could be as high as ~16%.

V. vulnificus causes a mild to severe gastrointestinal illness, potentially progressing to septicaemia with a significant mortality in a susceptible population. In the USA, mortality rates are between 50% and 60% for patients with *V. vulnificus* septicaemia (Hlady and Klontz, 1996; Shapiro et al., 1998). Septicaemia is the symptom with which patients typically present to health care systems. Thus in this risk assessment septicaemia was the endpoint modelled.

This risk assessment considers only reported cases of *V. vulnificus* septicaemia from 1995 to 2001 (mean of 32 cases annually) in which a history of consumption of raw Gulf Coast oysters is documented (M. Glatzer, personal communication, 2001). Because of the severity of the septicaemia, under-reporting is not as substantial a consideration (2:1) as with gastrointestinal illnesses, which the Centers for Disease Control (CDC) estimates to have a 20:1 under-reporting ratio (Mead et al., 1999). However, various sources of under-reporting of *V. vulnificus* septicaemia have been identified. Historically, FDA has only recorded cases where patients admitted eating oysters. Patients who ate oysters may have denied oyster consumption, may not have been willing to answer questions, or may have deceased before a food history could be obtained. Another source of under-reporting is the failure to capture all the cases in different reporting systems. Adjusting for such under-reporting indicate that there may be up to 2.5 times more *V. vulnificus* septicaemia cases associated with raw Gulf Coast oysters. While this risk assessment does not make any adjustment for possible under-reporting it would be possible to do this by shifting the dose-response relationship towards greater risk at a given dose (i.e. moving the dose-response curve to the left).

There are no human volunteer studies with *V. vulnificus* from which a dose-response relationship might be estimated. Data are available to estimate the relationship by comparing monthly exposure estimates for sensitive populations with monthly-observed epidemiological data in the USA using an approach similar to that proposed by Buchanan et al. (1997). A consistent *V. vulnificus* reporting system, administered by CDC, has been in effect since 1995 in the USA, and the CDC data is currently available through to 2001.

The month- and year-specific mean *V. vulnificus* numbers at consumption were estimated based on water temperatures from 1995 through to 2001. Monthly oyster landings were found to vary less substantially from year to year than temperature. Therefore, the number of servings each month was estimated assuming that 50% of the average landings (from 1990–1998) each month are consumed raw, and then converting to a corresponding number of meals based on average oyster weight and typical number of oysters per serving. The number of meals consumed by the susceptible population was estimated as being 7% of the total meals. For the purpose of dose-response modelling, the mean *V. vulnificus* dose per serving was used to summarize group level exposures rather than the median (or other statistic) because use of the mean has been shown to minimize bias of estimated dose-response relationships from group-level data under most circumstances (Crump, 1998). Also, it is the illness burden that is the public health impact of foremost concern, which, is in turn

determined by the mean (and not necessarily the median) risk per serving in respect to any given collection of (variable) exposures for which illness burden is of interest (see Appendix C). Figure 2 shows the maximum likelihood fit of the Beta-Poisson dose-response curve and the corresponding 90% confidence limits for risk of reported *V. vulnificus* illness. The dots represent the best estimates of the month- and year-specific risk per serving versus month- and year-specific estimates of average dose per serving based upon an analysis of exposure and observed epidemiology. The best estimates of parameters for the Beta-Poisson model are $\alpha = 9.3\times10^{-6}$ and $\beta = 110\ 000$.

RISK CHARACTERIZATION

With respect to the baseline assessment, using typical seasonal water temperature parameters, the predicted mean numbers of illnesses were 0.5, 11.7, 12.2 and 8.0 for the winter (January–March), spring (April–June), summer (July–September) and autumn (October–December) seasons, respectively. These predictions were based on the seasonal estimates of mean risk per serving and the estimated number of servings consumed, and are, therefore, necessarily consistent with the observed epidemiology. Based on the dose-response assessment, the effect of alternative process target levels on risk per serving and annual cases burden was evaluated

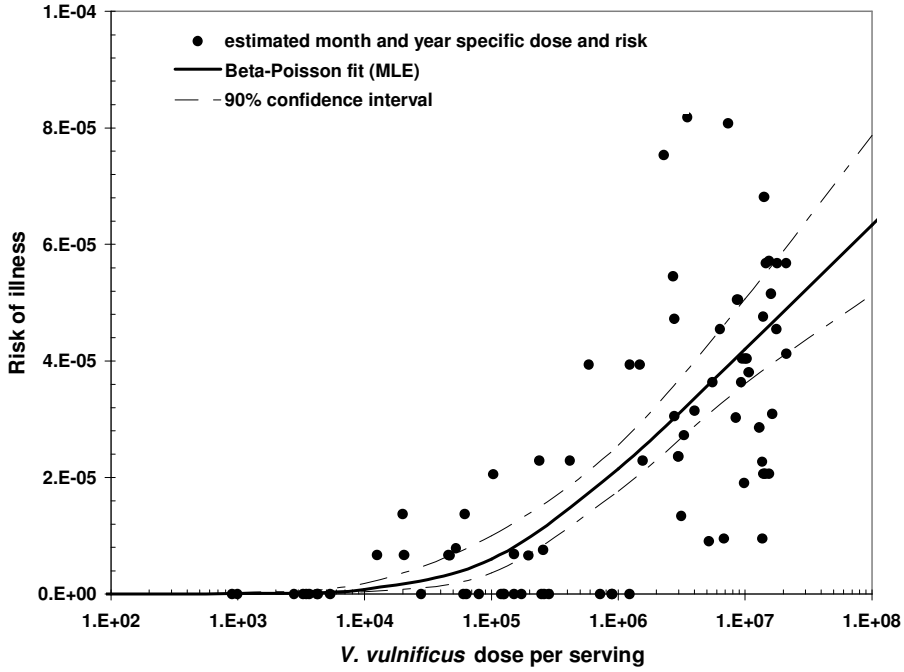

Figure 2. Beta-Poisson dose-response curve for *V. vulnificus* (Vv) (monthly average risk per serving versus monthly average dose per serving). Each data point is determined by risk of reported oyster-related illness (number of observed cases divided by estimated number of servings) and mean exposure corresponding to month- and year-specific water temperature data.

(Table 2). These target levels could be achieved by one or a range of intervention strategies. Substantial reductions in risk were found to be associated with target levels for *V. vulnificus* of 3/g and 30/g with an approximate 10-fold range of uncertainty. More details on the outcome and uses of this risk assessment are presented in the technical report.

Table 2. Predicted mean and 90% uncertainty intervals for risk per serving and annual number of illnesses for three alternative process target levels.

Target *V. vulnificus*/g	Mean risk per serving (median and 90% interval of uncertainty distribution)	Annual number of cases (median and 90% interval of uncertainty distribution)
3/g	1.09×10^{-7} (4.10×10^{-8}, 2.73×10^{-7})	0.16 (0.06, 0.4)
30/g	8.20×10^{-7} (3.42×10^{-7}, 2.12×10^{-6})	1.2 (0.5, 3.1)
300/g	5.26×10^{-6} (2.60×10^{-6}, 1.05×10^{-6})	7.7 (3.8, 15.3)

CONCLUSIONS

Key findings

- The utilization of the framework and parameters for the *V. parahaemolyticus* risk assessment facilitated the development of the *V. vulnificus* risk assessment.
- Where additional data were available it was possible to validate certain aspects of the risk assessment model. The exposure assessment predictions were validated by their close agreement with retail study data.
- In the absence of specific dose-response data it was possible to develop a dose-response relationship from exposure predictions and the reported frequency of illness which was effective for risk characterization and the evaluation of interventions.

Limitations and caveats

In terms of applying this risk assessment to other geographical areas there are a numbers of issues which need to be specifically considered and perhaps adjusted to take into account the local situation.

- The *V. vulnificus*-temperature relationship in oysters at harvest may not be applicable to other regions or countries with different environmental conditions, such as high salinity.
- Countries harvesting different species of oysters, and that have different post-harvest handling practices or consumption patterns, may have to adjust the model inputs to account for these differences.
- The proportion of susceptible individuals may be very different in some countries, such as those in Asia and Africa that have high prevalence of hepatitis C and HIV/AIDS, respectively, or in countries with high rates of alcoholism.

Some general limitations to this risk assessment were also identified. These were primarily linked to data limitations and the availability of additional data in the future may mean that certain aspects of the risk assessment would need to be revised.

- The dose-response relationship is based on predicted mean exposure and this was found to be sensitive to the choice of available data for the *V. vulnificus*-temperature relationship in oysters at harvest. Alternative data sets gave significantly different predictions of the variance of $\log_{10}$ densities even though the mean of $\log_{10}$ densities were similar. Since dose-response and risk characterization, by the methods adopted here, depend largely on mean values, representative estimates of population variance of $\log_{10}$ densities are necessary in order to estimate mean exposure.
- The dose-response assessment and risk characterization was based on the assumption that all strains are equally virulent and there are no seasonal or regional changes in virulence.

Conclusion and recommendations

The approach of extending the models developed in the FDA-VPRA and FAO/WHO-VPRA to model another pathogen, *V. vulnificus*, greatly facilitated the risk assessment process. The same framework and many of the model inputs were applicable for modelling *V. vulnificus* risk in USA oysters and sufficient data was available to conduct a useful risk assessment. It was also possible to develop the model in such a way as to evaluate the potential effectiveness of various mitigation strategies in terms of reducing *V. vulnificus* levels in oysters, and ultimately the risk of illness.

This model provides a strong basis for countries wanting to undertake a risk assessment on *V. vulnificus* in oysters. However, in order to apply the model it would be important to have data relevant to that country, particularly on *V. vulnificus* numbers in seafood's associated with primary septicaemia, at harvest and the point of consumption, and to characterize the susceptible population within that country. For seafood's other than raw oysters the model would need to be altered and evaluated, although the dose-response data may be applicable to other countries.

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TECHNICAL REPORT

1. Introduction

Vibrio vulnificus naturally inhabits warm estuarine environments and can infect humans via wound exposure or seafood consumption. These infections are rare and generally limited to individuals with pre-existing chronic illnesses or the immunocompromised. However, *V. vulnificus* can invade through the intestinal barrier into the bloodstream (primary septicaemia) and has the highest case/fatality rate (approx. 50%) among foodborne pathogens. While *V. vulnificus* has been found in a variety of seafood's worldwide and illnesses have been reported in a number of countries, its epidemiology, ecology and distribution in seafood's have been most extensively investigated in the United States of America (USA). Each year, 30 to 40 primary septicaemia cases are reported in the USA, and nearly all are associated with consumption of raw oysters harvested from the Gulf Coast.

A risk assessment for the pathogen-commodity pair of *V. vulnificus* in raw oysters was proposed by the European Community in the 33rd session of the Codex Committee on Food Hygiene (CCFH) (CAC, 2000). There have been at least two previous risk assessments on *V. vulnificus*. McCoubrey (1996) reported on the risk of *V. vulnificus* infection following consumption of raw commercially harvested North Island oysters from New Zealand in 1996. The report concluded that environmental conditions, especially high salinities, were not suitable for *V. vulnificus* survival. The European Commission's Scientific Committee on Veterinary Measures relating to Public Health has prepared a document on *V. vulnificus* and *V. parahaemolyticus* in raw and undercooked seafood (Scientific Committee on Veterinary Measures relating to Public Health, 2001). This work followed the general format of a risk assessment and noted variations in *V. vulnificus* prevalence on a global scale. However, neither of the above risk assessments was quantitative. In order to address the risk associated with *V. vulnificus* in a quantitative manner, within the framework of the FAO/WHO risk assessment on *Vibrio* spp. in seafood, it was decided to extend the *V. parahaemolyticus* models described in the U.S. Food and Drug Administration (USFDA) Draft Risk Assessment on the Public Health Impacts of *V. parahaemolyticus* in Raw Molluscan Shellfish ("FDA-VPRA") (FDA, 2001) and the Joint FAO/WHO Risk Assessment of *V. parahaemolyticus* in Raw Oysters ("FAO/WHO-VPRA") (FAO/WHO, in press) to *V. vulnificus.* The general approach proposed and many of the parameters used in the present *V. vulnificus* risk assessment are the same as those used in the FDA-VPRA and FAO/WHO-VPRA.

1.1. Scope

This risk assessment had several objectives. Firstly, it aimed to determine the usefulness of adapting the FDA-VPRA and FAO/WHO-VPRA models to assess the risk from *V. vulnificus* septicaemia associated with the consumption of raw oysters. Secondly, the risk assessment aimed to identify the most appropriate data, as well as gaps in the available dataset, for modelling purposes. In addition to estimating the risk of *V. vulnificus* septicaemia associated with the consumption of raw oysters, the risk assessment model was also developed with the objective of evaluating targeted mitigation levels aimed at reducing the risk of *V. vulnificus* septicaemia. For reasons of data availability, the risk assessment was limited to consideration

of primary septicaemia cases associated with consumption of raw oysters from the Gulf Coast of the United States of America (USA).

A number of factors facilitated the development of this risk assessment of *V. vulnificus* in raw oysters and served to reduce the amount of work needed to complete the risk assessment. These included the following:

1. A history of consistent active surveillance in the USA and a much higher reporting rate for primary septicaemia (>50%) compared with low and highly variable reporting for gastrointestinal infections.
2. A well characterized seasonal effect that relates exposure to frequency of *V. vulnificus* illness.
3. The dominance of a single vehicle of transmission (>90% of cases of illness are associated with the consumption raw Gulf Coast oysters).
4. The storage of oysters in the shell until consumption practically eliminates potential for cross-contamination, thereby reducing uncertainty.
5. Raw consumption eliminates variability and uncertainty on pathogen survival during preparation, in contrast to the diverse cooking procedures used with other commodities.
6. The availability of extensive quantitative data on levels of *V. vulnificus* in oysters at harvest and at the point of consumption.
7. Information on the growth and survival of natural *V. vulnificus* populations in oysters under typical industry practices from harvest to consumption.
8. The availability of the draft USFDA-VPRA (FDA, 2001) and the FAO/WHO-VPRA (FAO/WHO, in press), which provide a suitable framework and many of the necessary parameters formatted for risk assessment, thereby also reducing the work to be undertaken.
9. The tagging system for USA oysters which permits tracing of harvest data and locations.

The current risk assessment establishes a relationship for predicting *V. vulnificus* levels at harvest based on seasonal and yearly variations of water temperature. Distributions of storage times and temperatures representative of industry practices are used to predict growth and survival post-harvest. These distributions along with surveyed oyster consumption patterns are used to determine exposure to *V. vulnificus*. Predicted exposure was validated using data from a national market survey of *V. vulnificus* in raw oysters. Hazard characterization relied primarily on a dose-response relationship derived from the seasonal relationship of predicted exposure and reported illness frequencies. This dose-response relationship was used to predict illness frequencies at targeted mitigation levels that may be achieved through various interventions.

2. Hazard identification

Since *V. vulnificus* was first reported in the 1970s, it has been the subject of many research and review articles (Oliver, 1989; Strom and Paranjpye, 2000). Three biotypes of *V. vulnificus* have been reported (Bisharat and Raz, 1997; Bisharat et al., 1999): Biotype 1 accounts for nearly all human cases resulting from seafood consumption, whereas Biotype 2 is associated with infections in cultured eels and Biotype 3 has been limited to wound infections associated with handling fish cultured in inland ponds in Israel. Most of the studies of Biotype 1 *V. vulnificus* have been conducted in the USA, and outside of that country there is currently little epidemiological information as *V. vulnificus* is not a reportable disease in most countries and surveillance is limited. While foodborne *V. vulnificus* infections are relatively rare in the USA (approximately 30–40 reported cases of primary septicaemia per year), they have the highest case fatality ratio among foodborne illnesses, which exceeds 50% (Hlady and Klontz, 1996; Mead et al., 1999; M. Glatzer, personal communication, 2001). Individuals with pre-existing liver disease are at greatest risk of contracting primary septicaemia, with subsequent mortality, but other chronic illnesses and immune deficiency conditions are also associated with increased risk. Healthy individuals may be at risk for relatively mild gastroenteritis, which is outside the scope of this assessment, but the risk for primary septicaemia in the absence of reported risk factors is considered negligible.

A number of factors have been reported as possible virulence determinants in *V. vulnificus*, including an extracellular cytolysin, protease, siderophores, a phospholipase, polysaccharide capsule, resistance to bactericidal effects of human serum, resistance to phagocytosis, and the ability to acquire iron from transferrin (Oliver, 1989; Strom and Paranjpye, 2000). The relevance of these factors has been examined in various *in vivo* or *in vitro* models. Production of disease by this bacterium appears to be multifaceted, involving a variety of virulence attributes and host susceptibility factors. Most animal studies have not found major differences in virulence characteristics between clinical and environmental isolates of *V. vulnificus* (Table 1). However, this is inconsistent with the low attack rate in susceptible populations consuming seafood contaminated with *V. vulnificus*. Less than one illness occurs per 10 000 meals of raw Gulf oysters served to the highest risk population, people with liver diseases (Hlady, 1994), suggesting that environmental strains are not equally virulent or not all people with liver disease are equally susceptible.

Nearly all *V. vulnificus* primary septicaemia cases in the USA have been associated with consumption of raw oysters harvested from the Gulf Coast. These cases follow a seasonal distribution, with approximately 90% of cases occurring from April through November (M. Glatzer, personal communication, 2001). Only one confirmed foodborne case has been associated with oysters harvested other than from the Gulf Coast, and only two cases have been linked to oysters harvested in January or February. The seasonal numbers of *V. vulnificus* in Gulf Coast oysters at harvest (Motes et al., 1998; Tamplin, 1994) and at retail (Cook et al., 2002) exhibit a similar distribution to the cases of *V. vulnificus* illness.

Table 1. Summary of virulence testing from clinical and environmental *V. vulnificus* strains using various animal models.

Model	Dose/Route[3]	No. virulent/No. of isolates tested		Reference
		Clinical strains	Environmental strains	
Normal adult mouse	10^6/i.p.	2/3	Not tested	Poole and Oliver, 1978
Normal adult mouse	10^6/s.c.	2/3	Not tested	Poole and Oliver, 1978
Normal adult mouse	10^8/i.v.	1/1	Not tested	Poole and Oliver, 1978
Normal adult mouse	10^8/o.g.	0/1	Not tested	Poole and Oliver, 1978
Normal adult mouse	10^8/i.p.	20/20	25/29	Tison and Kelly, 1986
Normal adult mouse	10^8/i.p.	4/4	40/40	Kaysner et al., 1987
Normal adult mouse	10^6/i.p.	7/11	9/13	Stelma et al., 1992
Iron-overload mouse[1]	10^0/i.p.	1/1	Not tested	Wright, Simpson and Oliver, 1981
Iron-overload mouse[1]	10^3/i.p.	4/8	2/8	Morris et al., 1987
Iron-overload mouse[2]	10^2/i.p.	3/4	4/7	Kaysner et al., 1987
Iron-overload mouse[2]	10^2/i.p.	8/11	9/13	Stelma et al., 1992
Iron-overload mouse[2]	10^3/i.p.	1/1	1/8	Jackson, Murphree and Tamplin, 1997
Iron-overload and immuno-compromised mouse	10^3/i.p.	0/3	4/4	Stelma et al., 1992
Suckling mouse	10^6/o.g.	5/5	0/7	Johnson et al., 1984
Suckling mouse	10^7/o.g.	4/8	2/8	Morris et al., 1987
Suckling mouse	10^5/o.g.	5/6	2/5	Reyes et al., 1987

NOTES: (1) Ferric ammonium citrate (80 mg/kg) used to produce iron-overload. (2) Iron dextran (250 mg/kg) used to produce iron-overload. (3) Route of administration (i.p. = intraperitoneal injection; s.c. = subcutaneous injection; i.v. = intravenous injection; o.g. = orogastric ingestion).

3. Exposure assessment

3.1. Microbial ecology

Vibrio vulnificus is a bacterium that occurs naturally in estuaries in many parts of the world (Oliver, 1989). Its distribution and abundance is affected by temperature and salinity of the seawater. *V. vulnificus* is present in waters, sediments, plankton, molluscs, crustaceans and finfish estuaries of the Gulf Coast of the USA (Tamplin, 1990; DePaola, Capers and Alexander, 1994). A recent study in India highlighted the presence of *V. vulnificus* in the tropical waters of the southwest coast of India (Parvathi et al., 2004).

Attention is generally focused on oysters, since most *V. vulnificus*-related foodborne illnesses in the USA are linked to their raw consumption. *V. vulnificus* is found in various tissues of the oyster and may reside within oyster haemocytes (Tamplin and Capers, 1992; Harris-Young et al., 1993). Each oyster may shed up to one million *V. vulnificus* cells per day into the water, demonstrating its ability to multiply within the oyster (Tamplin and Capers, 1992).

Typically, USA Gulf Coast oysters harbour about 1000 *V. vulnificus* cells per gram during the warmer months of April through to October, and usually less than 10 per gram during other months, although *V. vulnificus* may become undetectable in Gulf Coast oysters during unusually cold periods (DePaola, Capers and Alexander, 1994; Motes et al., 1998). Oysters from the southwest coast of India were found to harbour *V. vulnificus* at a level of 1000 per gram when water salinity was at its lowest, during and after the monsoon (Parvathi et al., 2004). At other times of the year, when water salinity was more than 25 ppt, less than 10 cells per gram were detected. Some evidence suggests that this bacterium lives in oysters year round but may become dormant or viable but non-culturable (VBNC) during cold weather; a temporary condition reversible by increasing water temperature (Nilsson, Oliver and Kjelleberg, 1991). *V. vulnificus* numbers in seawater are approximately 100-fold lower than in oysters, but numbers frequently exceed 10^6 per gram in the intestines of bottom feeding fish that inhabit oyster reefs (DePaola, Capers and Alexander, 1994).

Unlike many shellfish-borne human pathogens, *V. vulnificus* is not associated with human faeces and traditional indicators of faecal pollution (i.e. faecal coliforms) are not effective at predicting its abundance in oysters (Tamplin et al., 1982). A number of factors may interact with temperature and salinity to control *V. vulnificus* populations in oysters, including nutrient availability, resuspension of sediments, plankton blooms, defecation by vertebrates (i.e. finfish), phagocytosis by oyster haemocytes, competition, predation, phage infections and a variety of physical factors (pH, dissolved oxygen, water chemistry and sunlight). The effect of these factors on *V. vulnificus* ecology is unknown, making the prospect of developing a reliable indicator model in the near future extremely unlikely.

3.2. Growth and survival characteristics

The bacterium may grow at temperatures as low as 13°C (Kaspar and Tamplin, 1993), but its numbers in the environment remain low at temperatures below 20°C (Kelly, 1982; O'Neill, Jones and Grimes, 1992). Highest concentrations occur when the water temperature is between 20°C and 30°C. Thus, *V. vulnificus* is more abundant along the Gulf Coast than in the cooler waters of the Atlantic and Pacific Coasts of the USA (DePaola, Capers and Alexander, 1994; Cook, 1994; Tamplin, 1990; O'Neill, Jones and Grimes, 1992; Kaysner et al., 1987; Motes et al., 1998). *V. vulnificus* can be found at salinities ranging from 0.8 to 35 ppt (Tamplin, 1990; Kaysner et al., 1987). The salinity optimum for *V. vulnificus* appears to vary considerably from area to area, but highest numbers are usually found at intermediate salinities of 5 to 25 ppt (Tamplin et al., 1982; Kelly, 1982; O'Neill, Jones and Grimes, 1992; Tamplin, 1990; Motes et al., 1998).

V. vulnificus is more sensitive than other *Vibrio* spp. and most other foodborne pathogens to most inactivation techniques used in food processing. A mild heat treatment of 50°C for 5 minutes yielded a 6 $\log_{10}$ reduction in *V. vulnificus* in shucked oyster meats (Cook and Ruple, 1992). Freezing oysters at -40°C and storage for 3 weeks achieved a 4 to 5 $\log_{10}$ reduction in the natural *V. vulnificus* population (Cook and Ruple, 1992). However, the effectiveness of freezing may be reduced in *V. vulnificus* cells subjected to a cold adaptation step of 15°C prior to freezing (Bryan et al., 1999). Similar reductions can be readily achieved by irradiation (Ama, Hamdy and Toledo, 1994) and high hydrostatic pressure (Berlin et al., 1999). Low pH is quite lethal to *V. vulnificus* (Koo, Marshall and DePaola, 2001), but organisms within the oyster tissues may be protected from acidic hot sauces and other chemicals as these would probably not penetrate to the interior of an oyster (Sun and Oliver, 1994, 1995). Depuration was shown not to be effective in elimination of *V. vulnificus* as it resides within various oyster tissues, but relaying oysters to high salinity waters (>32 ppt) was shown to reduce *V. vulnificus* numbers by 3–4 logs (<10 per g) within 2 weeks (Motes and DePaola, 1996).

3.3. Consumption of oysters

Intake data for molluscan shellfish are available from a number of governmental and non-governmental sources, but the information in the data is sometimes limited. In many countries there is a scarcity of such consumption data, as noted recently in the European Union (Scientific Committee on Veterinary Measures relating to Public Health, 2001). Because raw shellfish is not a commonly consumed food in many countries, including the USA (10–20% of the population consumes shellfish raw at least once during a year), the available data from nationwide general nutrition surveys generally do not provide definitive information as to consumption patterns. For example, data from the U.S. Department of Agriculture (USDA) Continuing Survey of Food Intake by Individuals (CFSII) (USDA/ARS, various dates) suggest that, over the aggregate USA population, raw oysters are consumed on average at a rate of 0.0005 servings per person per day, with a mean serving size of 110 grams. While this rate of consumption is based on a relatively large number of survey respondents (~15 000), the estimate of serving size from the same data is much more uncertain. It is based on the consumption behaviour of only 6 individuals reporting consumption during the survey period.

Another food frequency survey, which was specific to raw oyster consumption in the state of Florida in the USA, was conducted by the Agricultural Market Research Center, University of Florida (Degner and Petrone, 1994). This study was judged to provide

substantially better information with respect to the probable distribution of serving sizes and, though regional, was considered more appropriate as an estimate of the national consumption patterns, bearing in mind the limited number of individuals reporting oyster consumption in the larger (national) surveys. The data from the Florida survey indicate a distribution of serving sizes with a mean of ~13.7 oysters/serving. Combining these data with oyster weight data obtained during a retail study (Cook et al., 2002; A. DePaola, personal communication, 2002) suggests a mean serving size of ~196 g. Based on this estimate of average serving size, the total number of servings consumed can be determined from landings data (NMFS, 1998) and an estimate of the percentage of the harvest that is consumed raw.

This is the approach to estimation of consumption behaviour (number of servings and size of serving) that was used in the FDA-VPRA and the FAO/WHO-VPRA and was taken from that work for the purpose of this assessment. Since the current risk assessment is restricted to consideration of risk associated with USA Gulf Coast oysters, only oysters harvested from that specific region were considered here.

3.4. Modelling exposure to *V. vulnificus*

A schematic diagram of the *V. vulnificus* risk assessment model is shown in Figure 1. Modelling exposure to *V. vulnificus* followed the same approach and used many of the same assumptions as used for the FAO/WHO-VPRA (FAO/WHO, in press) and FDA-VPRA (FDA, 2001). These are summarized in Table 2. While foodborne *V. vulnificus* infections have been reported in countries other than the USA, such as Taiwan (Chuang et al., 1992) and the Republic of Korea (Park, Shon and Joh, 1991), sufficient data are currently available only in the USA for most of the model inputs shown in Figure 1. This framework could be used by other countries to model the risk of *V. vulnificus* illness from raw oysters when the appropriate data is available. It may also be modified to address other seafood's.

The production-process-retail-consumption continuum was modelled using a modular approach. Each of the modules – harvest, post-harvest including storage, and retail – and the data sources for each are outlined in the following sections. For each identified step in the process continuum, variability distributions or quantitative relationships, or both, between variables were estimated based on the available data. Where applicable, uncertainties associated with these distributions and relationships were quantified. The estimated variability and uncertainty distributions and their interdependences were then implemented using Monte Carlo modelling software in order to calculate model-based exposure predictions and associated uncertainties (see Appendix A).

3.4.1. Harvest

3.4.1.1. Water temperature and salinity distributions

Like *V. parahaemolyticus*, the numbers of *V. vulnificus* at harvest are influenced predominantly by water temperatures and salinities. Other factors may also contribute to *V. vulnificus* numbers, but only the effects of temperature and salinity have been quantified (Motes et al., 1998).

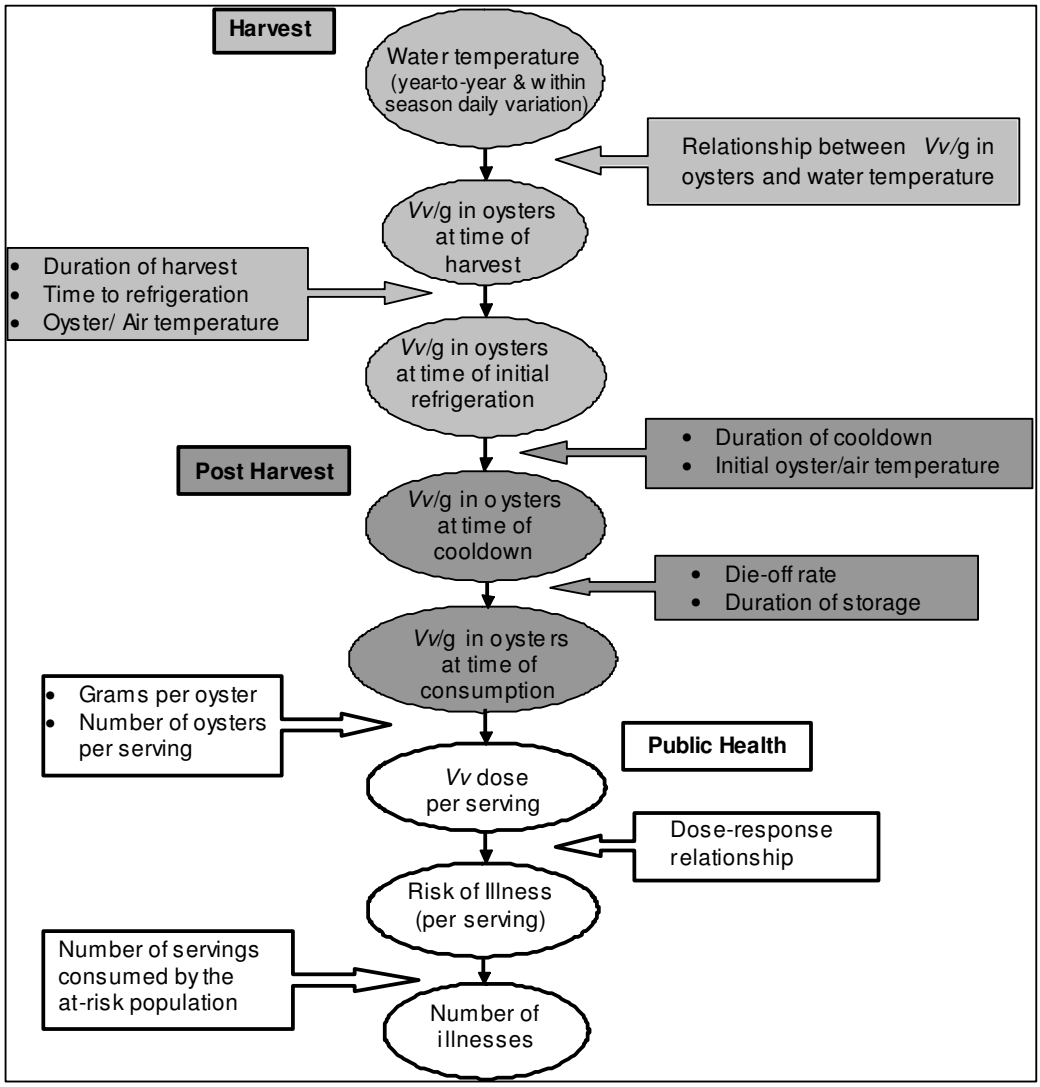

Figure 1. Schematic diagram of the *V. vulnificus* (Vv) conceptual risk assessment model, showing integration of all modules.

Table 2. Model inputs, data sources and assumptions for the proposed *V. vulnificus* risk assessment

Model Inputs	Data Source	Assumptions
Water temperature	FAO/WHO-VPRA/FDA-VPRA; NOAA buoy data (NOAA, 1999); and NERRS (NERRS, 2001)	Buoy and other fixed site data are representative of growing areas.
Total *V. vulnificus* numbers at harvest	Weekly oyster samples from 4 Gulf States 1994–1995 (Motes et al., 1998; Tamplin, 1994)	Data relevant for other years.
Pathogenic *V. vulnificus* numbers at harvest	Weekly oyster samples from 4 Gulf States 1994–1995 (Motes et al., 1998; Tamplin, 1994)	All *V. vulnificus* strains are equally virulent. Data from Jackson, Murphree and Tamplin (1997) and two recent studies (Nilsson et al., 2003; DePaola et al., 2003) suggest opposing view on this subject, but neither addresses seasonal or regional differences in virulence.
Air temperature	FAO/WHO-VPRA/FDA-VPRA; NOAA buoy data (NOAA, 1999)	Temperature of oyster meat equilibrates rapidly to that of air.
Time harvest vessel in water	FAO/WHO-VPRA/FDA-VPRA; Dealer survey (Cook, 1997b)	Harvest practices have not changed since 1996.
Time to first refrigeration	FAO/WHO-VPRA/FDA-VPRA; Dealer survey (Cook, 1997b)	Oysters are harvested at a constant rate throughout the harvest period.
V. vulnificus growth rate	Natural populations in oysters at ambient temperature (Cook, 1997a). Data lacking at lower temperature	*V. vulnificus* grows at similar temperature-specific rates in Gulf oysters from April to October.
V. vulnificus/g at first refrigeration	Dealer survey of Gulf oysters 1995–1996 (Cook, 1997b)	Dealer practices in 1996 are typical of current practices.
Cool down time	FAO/WHO-VPRA/FDA-VPRA; no data	Rectangular (uniform) distribution between 1 and 10 h.
V. vulnificus/g at cool down	Dealer survey of Gulf oysters 1995–1996 (Cook, 1997b)	Dealer practices in 1996 are typical of current practices.
V. vulnificus survival	Natural populations in oysters stored at 3°C for 14-17 d (Cook et al., 2002)	*V. vulnificus* die off at 3°C similar to that at other temperatures between 0-13°C.
Pathogenic *V. vulnificus*/g at consumption	Retail study of USA oysters 1998–1999 (Cook et al., 2002)	Data are relevant for other years. (The autumn of 1998 was extremely warm and *V. vulnificus* levels were considerably higher than levels predicted for typical autumn temperatures).
Percentage of population susceptible	Prevalence of liver disease, immune disorder, etc., in the USA (Klontz, 1997; Desenclos et al., 1991; Shapiro et al., 1998; Hlady, 1997)	All predisposing conditions have been identified and risk of infection is homogeneous with respect to these conditions. There is no risk of illness (septicaemia) to individuals without the identified disease conditions.
Percentage of oysters consumed raw	50% oysters consumed raw; FAO/WHO-VPRA	Susceptible individuals consume raw oysters at the same rate as the total population.
No. of oysters per serving	FAO/WHO-VPRA/FDA-VPRA	Consumption behaviour has not changed appreciably in recent years.
Weight per oyster	Retail study of USA oysters 1998–1999 (Cook et al., 2002)	None.
Risk of illness	Relationship between monthly exposure and illness within defined (Gulf States) population (Cook et al., 2002; M. Glatzer, personal communication, 2001; NMFS, 1998)	Consumption of raw oysters among the susceptible population does not vary from month to month. (If the percentage of raw oysters consumed was greater in cooler months, the effect of the assumption would be to over-predict the risk at lower *V. vulnificus* levels). Reporting of *V. vulnificus* septicaemia cases related to oyster consumption does not vary from month to month.
Number of illnesses	Risk of illness (from above) multiplied by number of servings	Same as above for risk of illness.

The FAO/WHO-VPRA did not quantify the distribution of salinities prevalent in growing areas. It was noted that there was little systematic collection of salinity data outside of a few selected estuaries (e.g. sites within the National Estuarine Research Reserve System – NERRS – within the USA), and that what data was available was relatively recent and did not quantify extremes of salinities that might be expected over longer periods. Water temperature data was obtained from the National Buoy Data Center (NOAA) for sites considered representative of selected regions in the USA. For the purposes of this exposure assessment, the FAO/WHO-VPRA and FDA-VPRA water temperature distributions for the USA Gulf Coast based on the NOAA data were used. However, this was supplemented by additional data from a nearby NERRS site (NERRS, 2001) for selected periods during which, due to instrument malfunction, data was not available from the NOAA temperature record.

Seasonal exposure assessments were performed for the Gulf Coast. The water temperature in the summer averages 28.9°C and varies from day to day, with a standard deviation of 1.5°C. The corresponding average and standard deviation in the winter are 14.2°C and 2.7°C, respectively. Spring and autumn are typically transitional periods with greater month-to-month variability. The temperature parameters (mean and standard deviation of day-to-day variations) within any given season vary from year to year, as discussed in the FAO/WHO-VPRA, and this variation was incorporated into the present exposure assessment.

The effect of salinity on predicted *V. vulnificus* numbers merits particular consideration. Effects of salinities on *V. vulnificus* may be stronger than on *V. parahaemolyticus* and lack of comprehensive season-specific and harvest-area-specific salinity data presents a potential data gap. While salinities >30 ppt are unusual in commercial oyster growing areas along the USA Gulf Coast, they are typical of many growing areas both in other parts of the USA and many other countries and are generally associated with low or non-detectable levels of *V. vulnificus* regardless of temperature.

There appears to be a threshold salinity level (i.e. at or slightly above 30 ppt) at which point *V. vulnificus* levels drop substantially, regardless of temperature. This abrupt change in *V. vulnificus* levels relative to salinity and the observation of a large proportion of non-detectable levels at high salinities makes quantitative estimation of the joint effects of salinity and temperature problematic over the entire range of both moderate and high salinity. Consequently, prediction of *V. vulnificus* levels has been addressed separately for high versus moderate (or low) salinity. It should be noted that the incremental effect of salinity within the moderate salinity range (i.e. <30 ppt) is not, ostensibly, the same as the effect at high salinity.

3.4.1.2. Prediction of the distribution of at-harvest *V. vulnificus* numbers at moderate salinities (<30 ppt)

Levels of *V. vulnificus* in USA Gulf Coast oysters at harvest have been investigated in a number of studies (Tamplin et al., 1982; Motes et al., 1998; Vanoy, Tamplin and Schwartz, 1992; Jackson, Murphree and Tamplin, 1997). The study by Motes et al. (1998) examined *V. vulnificus* numbers in three major Gulf Coast estuaries at weekly intervals for 15 months and was selected for the exposure assessment as samples were collected more intensively and extensively than in previous studies (Motes et al., 1998 – hereafter referred to simply as the Motes et al. study). The enumeration procedures used in this study were the same as those used for other model inputs in this exposure assessment such as examinations of market oysters for levels of the pathogen and determining *V. vulnificus* growth and survival.

Additional data on *V. vulnificus* levels in oysters at harvest were provided to the risk assessment drafting group by Dr Mark Tamplin. These data were collected weekly or fortnightly from selected sites on the Gulf, Pacific and Atlantic Coasts from 1994 and 1995. The method of enumeration was comparable to that of the Motes et al. study. *V. vulnificus* levels in samples collected from Gulf Coast sites in the Tamplin (1994) study were found to be generally consistent with the observations of Motes et al. in regard to mean $\log_{10}$ levels but with substantially more variability around the mean $\log_{10}$ levels. The data collected outside of the Gulf Coast were from relatively cold water sites and *V. vulnificus* levels were frequently found to be below the limit of detection (0.3 MPN/g). These data were consistent with the observed *V. vulnificus* levels in Gulf Coast samples collected at similarly low temperatures but, due to the censoring at the limit of detection, these data provide limited information with respect to estimating a quantitative relationship between *V. vulnificus* levels and water temperature.

Like *V. parahaemolyticus*, ambient water temperatures and salinities influence *V. vulnificus* numbers at harvest (Oliver, Warner and Cleland, 1982; Motes et al., 1998). Correlation statistics indicate that temperature and salinity account for about 60% to 70% of the total variation of observed *V. vulnificus* numbers (Motes et al., 1998). The effect of salinity was found to be significant, with the R^2 of a temperature-only regression model being 0.60 in comparison with an R^2 of 0.70 when the effects of salinity were added to the regression model (Motes et al., 1998). This suggests that, after temperature, effects of salinity explain an additional 10% of the total variation of *V. vulnificus* numbers observed in the USA Gulf Coast. Other factors may influence the regional and seasonal variation but there is insufficient information available at present for the purpose of quantitative modelling.

Given the limited information in the Tamplin data collected outside of the Gulf Coast, only the data collected from Gulf Coast sites was evaluated further. These data (referred to hereafter as the "Tamplin study data") were considered together with the data from the Motes et al. study to evaluate and estimate the effects of temperature (and salinity) on *V. vulnificus* levels. Following the approach of Motes et al., standard (so-called "fixed effect") polynomial regression models were used. These models assume homogeneous residual variation around fitted regression lines. Consequently, given that *V. vulnificus* levels are observed to be unevenly distributed (i.e. positively skewed) densities were log-transformed to normalize the variance and obtain a response variable consistent with the modelling assumptions underlying use of these standard regression methods.

Additionally, in the Tamplin data, *V. vulnificus* was frequently non-detectable at low to moderately low temperatures (i.e. near or below 15°C). Such measurement outcomes are said to be censored at the limit of detection and "censored"-regression likelihoods are appropriate for obtaining parameter estimates from such data when the proportion of non-detects is high or otherwise unduly influential. Given the frequency of non-detects observed in the Tamplin data at low temperature, this approach was judged to be appropriate and was adopted here. In comparison, very few samples in the Motes et al. study had non-detectable levels. On only two occasions were the levels of *V. vulnificus* below the limit of detection in each of two replicate samples analysed per sampling occasion. When only one of the two replicates had non-detectable levels in the Motes et al. study, the MPN estimate of the sample with a detectable level was taken as the estimate corresponding to the sampling occasion, and the geometric mean was used when both replicates had detectable levels. On the two occasions when both replicates had non-detectable levels, the level corresponding to the sampling

occasion was considered censored. The limit of detection for both the Tamplin and the Motes et al. studies was 0.3 MPN/g.

The polynomial regression model(s) used to examine and summarize temperature effects based on the two available data sets was of the general (quadratic) form:

$$\log(Vv/\text{g}) = \alpha + \beta_1 * Temp + \beta_2 * Temp^2 + \varepsilon$$
$$\varepsilon \sim Normal(0,\sigma^2)$$

In this equation the parameters α, β_1, and β_2 determine the regression line (or curve) and ε is the random residual (unexplained) variation, assumed to be independent of temperature and normally distributed with variance σ^2. When considering effects of salinity (below), a similar quadratic effect (in salinity) was added to the temperature-only model (above). Statistical significance of parameter estimates were examined to determine whether or not the quadratic model provided a significant improvement over the usual linear model (i.e. β_2 equal to zero).

In the process of evaluating the appropriateness of the fit of models to the available data, the model was fitted to the Motes et al. and Tamplin data sets separately; and then to the Motes et al. and Tamplin data combined (see Appendix B). Comparison of the results of these model fits to the data sets separately and combined indicated substantial differences, which could not be reconciled in the context of the regression model being used to summarize the information in these data (see Appendix B). This finding indicated that it was not appropriate to simply combine data, on an equal basis, into a single (pooled) data set within the context of the selected model(s). The implications of this can be somewhat problematic from a modelling or statistical perspective. As noted by one reviewer, when data sets are pooled and discrepancies are then identified in statistical analyses, this may be taken as an indication that the model being fitted to the data is mis-specified. That is, one may be able to identify other types of equally plausible models that effectively explain and account for the appearance of divergent characteristics in data obtained from different studies. Such statistical models and methods, when applied to data combined from multiple studies, are generally referred to as being "meta-analyses".

As is the case with all types of statistical analysis, meta-analysis of pooled data to model (or estimate) common effects (i.e. temperature) and study-specific effects should be grounded on a plausible rationale. That is, models selected to summarize the data or to account for apparent differences between studies or both, should be sensible and understandable. However, given limited information, this can not always be satisfactorily achieved. This, it was judged, is the case here. A plausible and sensible "meta-analytic" model that would explain the apparent and substantial differences between the Tamplin versus Motes et al. data sets was not identified. In the absence of such a model, the divergence of results obtained from these multiple analyses with different data sets (i.e. separately versus pooled) was considered an uncertainty and this uncertainty was propagated through the risk assessment (see Appendix D) to fully evaluate its effect. The outcome of comparing these results indicated that exposure predictions based on the Motes et al. data were more consistent with data available for validation, and this is discussed further below, as well as in Appendix D. Thus, it was concluded that the "best estimate" of the relationship between water temperature and *V. vulnificus* levels in oysters at harvest was associated with estimates obtained by analysis of the Motes et al. data alone.

The maximum likelihood estimate (MLE) of a temperature-only regression of the quadratic form based on the Motes et al. Gulf Coast data alone is shown in Figure 2. The data

shown in the figure are the averaged MPN determinations of the two replicate samples collected per each sampling occasion during this study.

In fitting the model to these data, the quadratic effect (β_2) was found to be a significant improvement over that of a linear regression model, as there is evident plateauing of *V. vulnificus* levels at temperatures above 25°C. Predictions of mean $\log_{10}$ *V. vulnificus* numbers based on this fitted regression were judged appropriate in the range of temperature from 10°C up to ~32°C (i.e. up to but not beyond the maximum of the quadratic). Similar analysis of the Tamplin data alone indicated that the quadratic (i.e. plateauing) effect at higher temperatures was not significant (see Appendix B). The MLEs of the parameters of the fit to the Motes et al. data are:

$$\alpha = -5.66$$
$$\beta_1 = 0.56$$
$$\beta_2 = -0.0086$$
$$\sigma = 0.73$$

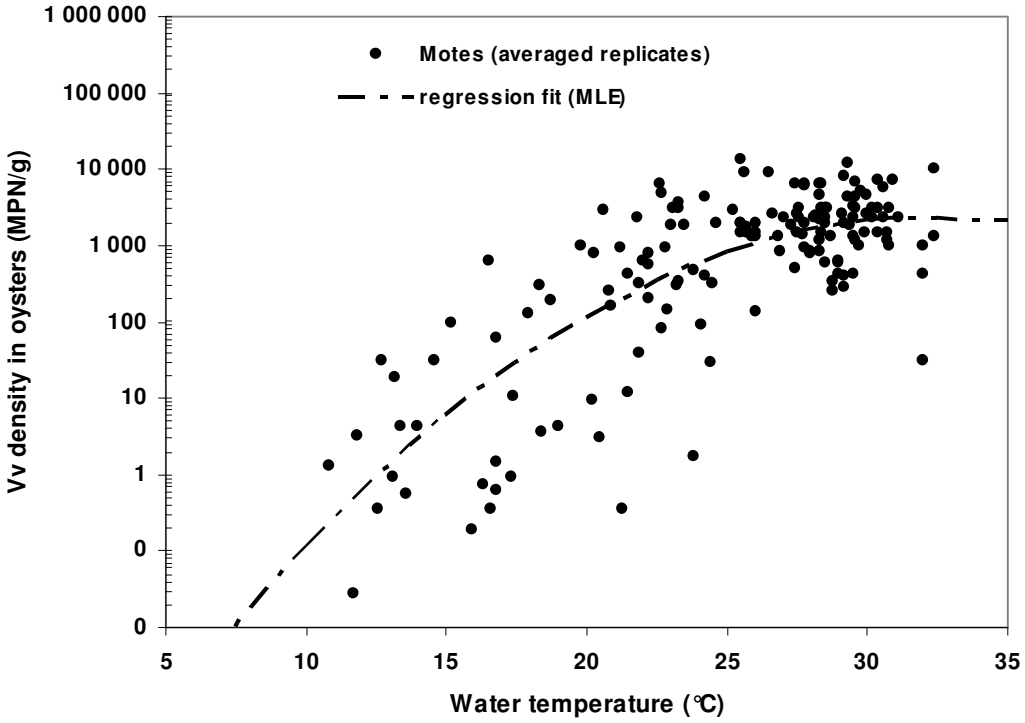

Figure 2. *Vibrio vulnificus* (Vv) numbers in USA Gulf Coast Oysters. Data from Motes et al., 1998.

The estimates of regression parameters based on fitting the same model to the Tamplin data set alone and then to pooled data from both studies are given in Appendix B. Additionally, with respect to all of these analyses, the uncertainty associated with the MLEs of the parameters of the regression model fits was characterized by the asymptotic variance-covariance of the parameter estimates. This is a large sample "normal approximation" of the uncertainty of the parameter values consistent with the observed data. This approximation was judged appropriate given 160 sampling occasions (and duplicate samples per sampling occasion) in the Motes et al. study and a comparable number of sampling occasions in the Tamplin study. Thus, when incorporating model estimates of the temperature effect into model simulations of exposure, the variance-covariance matrices of the parameter estimates were used (together with the MLEs) to generate uncertainty samples of parameter values consistent with the data. This uncertainty was then propagated through the assessment.

In the application of the parameter estimates for exposure prediction, the estimate of variance about the regression mean (σ^2) merits particular attention in so far as the estimated value includes the effects of both: (1) the natural (i.e. "true" or actual) variation of *V. vulnificus* densities independent of temperature effects, and (2) the method error attributable to a 3-tube MPN procedure. The natural component of the residual (unexplained) variation represents real variation due to effects other than temperature, such as that associated with variations in salinity and other, as yet unidentified factors. The method error component of the variation is artefactual and an estimate of this must be subtracted from the total residual variation in order to obtain an estimate of the natural variation. For the 3-tube MPN procedure, the method error has been estimated to be 0.12 (DePaola et al., 1997). Consequently, an estimate of the natural (population) variation of $\log_{10}$ *V. vulnificus* per gram (in composites of 12 oysters) was obtained by subtracting 0.12 from the estimated total observed residual variation (σ^2). For example, the MLE of the total variation is 0.73^2 based on the Motes et al. data (after averaging replicate measurements per sampling occasion). This estimate corresponds to the mode of the uncertainty distribution of values for this parameter. The corresponding mode of the inferred uncertainty distribution of values for the natural variation is 0.64 (i.e. 0.73^2 - 0.12) with similar calculations applying to other percentiles of the uncertainty distribution. The same method error was assumed to apply to both the Motes et al. and Tamplin data sets.

Although not incorporated in the present assessment, Motes et al. found that salinity had an appreciable effect on *V. vulnificus* numbers in the Gulf of Mexico. Based on a combined temperature and salinity regression model that is quadratic for both of these factors, Figure 3 illustrates the effect of ignoring salinity and predicting *V. vulnificus* numbers based on the temperature alone for these data. The temperature and salinity regression indicates that optimal salinity for *V. vulnificus* was approximately 17 ppt. For salinities between 12 and 24 ppt, ignoring the effect of salinity will at most over-predict $\log_{10}$ *V. vulnificus*/g by 10% when water temperature is greater than 25°C. However, extremes of salinities, i.e. <10 or >25 ppt, can be detrimental to *V. vulnificus* survival, and predictions of *V. vulnificus* numbers at these extremes based on temperature alone may over-predict by >20% depending upon the temperature. The potential for over-prediction is more pronounced at lower water temperatures.

It should be noted that these predictions assume a continuous (quadratic) effect of salinity on *V. vulnificus* levels. The quadratic model is valid only within a range of salinities about the central (optimal) salinity level. As already indicated, based on comparison of *V. vulnificus*

levels observed in high versus moderate salinity regions, there is an apparent abrupt change of the salinity effect at or slightly above 30 ppt. Consequently, the quadratic model of salinity effect can not be used to extrapolate across the apparent discontinuity and the effect of high (>30 ppt) salinity is addressed separately.

3.4.1.3. Prediction of the distribution of at-harvest *V. vulnificus* numbers at high (>30 ppt) salinities

While salinities >30 ppt were rarely observed by Motes et al. or Tamplin in Gulf Coast oyster growing waters they were typical of growing areas sampled on the Atlantic coast of the USA (North and South Carolina) (Motes et al., 1998). The *V. vulnificus* levels in most oyster samples were non-detectable when salinity exceeded 30 ppt, regardless of temperature (Table 3). Similar results were obtained from a study in Tokyo Bay in Japan (Table 4) and a recent study in India (Parvathi et al., 2004). This may be an important consideration in countries such as New Zealand that have a major portion of their oyster production in high salinity waters.

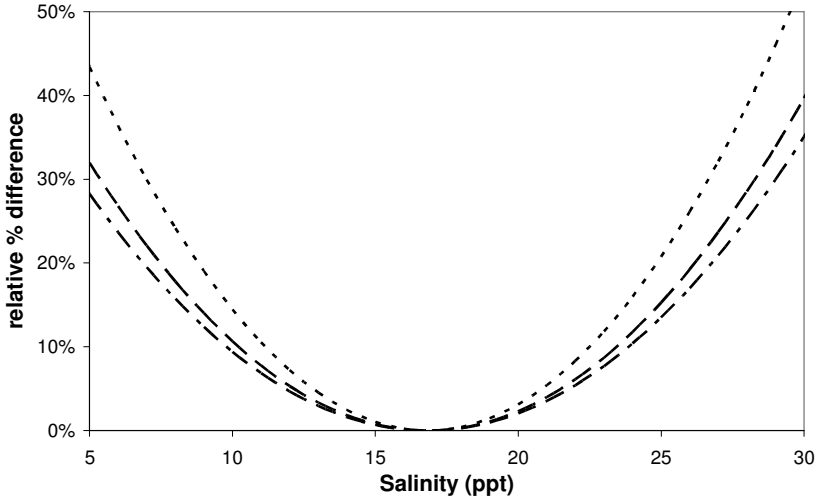

Figure 3. Differences in predictions obtained from a temperature-only compared with a temperature and salinity model for $\log_{10}$ *V. vulnificus* in Gulf Coast oysters at harvest. This relationship may not be applicable to other areas or species.

– – – – – – – Relative difference between models at temperatures of 20°C
—— —— —— Relative difference between models at temperatures of 25°C
—— — —— — Relative difference between models at temperatures of 30°C

Table 3. Effect of high (>30 ppt) versus moderate (<30 ppt) salinity on the frequency of detection of *V. vulnificus* and estimates of mean *V. vulnificus*/g in oysters sampled from selected USA Atlantic and Gulf Coast harvest areas (Motes et al., 1998).

Salinity range	Temperature range	No. of samples	Samples with detectable *V. vulnificus* levels	Mean *V. vulnificus*/g[(1)]
<30 ppt	<20°C	86	62.8%	140
<30 ppt	20–25°C	87	90.8%	1360
<30 ppt	25–30°C	157	96.2%	3120
<30 ppt	>30°C	50	96.0%	3170
<30 ppt	all	380	87.4%	2050
>30 ppt	< 20°C	22	40.9%	2.8
>30 ppt	20–25°C	33	30.3%	19.5
>30 ppt	25–30°C	30	23.3%	2.7
>30 ppt	>30°C	14	35.7%	4.2
>30 ppt	all	99	31.3%	8.5

NOTES: (1) Mean values calculated by averaging *V. vulnificus*/g (Vv/g) after imputing half the limit of detection (LOD) for samples with <0.3 MPN/g or <3 MPN/g; estimates are likely to be biased high as a consequence of imputing half the limit of detection (LOD) for non-detectable outcomes and failure to correct for the presence of method error.

Table 4. Effect of high (>30 ppt) versus moderate (<30 ppt) salinity on the frequency of detection of *V. vulnificus* and estimates of mean *V. vulnificus*/g in oysters sampled from Tokyo Bay, Japan (Oonaka et al., 2002).

Salinity range	Temperature range	No. of samples	Samples with detectable *V. vulnificus* levels	mean *V. vulnificus*/g[(1)]
<30 ppt	<20°C	0	–	–
<30 ppt	20–25°C	402	4.48%	0.16
<30 ppt	25–30°C	749	50.20%	86
<30 ppt	>30°C	142	90.85%	1116
<30 ppt	all	1293	40.45%	172
>30 ppt	< 20°C	43	0.00%	0.15
>30 ppt	20–25°C	364	8.52%	0.31
>30 ppt	25–30°C	156	50.64%	32.98
>30 ppt	>30°C	0	–	–
>30 ppt	all	563	19.9%	9.3

NOTES: (1) Mean values calculated by averaging *V. vulnificus*/g (Vv/g) after imputing half the limit of detection (LOD) for samples with <0.3 MPN/g; estimates are likely to be biased high as a consequence of imputing half the limit of detection (LOD) for non-detectable outcomes and failure to correct for the presence of method error.

Unfortunately, the high prevalence of non-detectable levels of *V. vulnificus* severely complicates efforts to quantitatively model effects of temperature and salinity in high salinity regions. Furthermore, given the apparent abrupt change in *V. vulnificus* levels at (or near) 30 ppt, the relationship between *V. vulnificus* levels versus temperature and salinity in high salinity regions can not be inferred from data obtained in moderate (or low) salinity regions. Consequently, the distribution of at-harvest *V. vulnificus* numbers in high salinity regions was not quantitatively modelled. Rather, based on imputing conservative estimates for non-detectable samples, worst case estimates of harvest densities were obtained based on the

Motes et al. and the Tokyo Bay data (Oonaka et al., 2002). The effect of temperature was examined by partitioning the data into temperature ranges. A similar treatment of the data at moderate or low salinity (<30 ppt) is given in Tables 3 and 4 for comparison with the results at >30 ppt.

When salinity was >30 ppt, 69% of USA samples and 80% of Tokyo Bay samples were less than the limit of detection (generally 0.3 MPN/g). Overall, conservative estimates of mean levels at >30 ppt were comparable with estimates of 8.5 and 9.3 *V. vulnificus*/g based on the USA and Japanese data, respectively. No temperature effect was evident in the USA data at high salinity but this was probably a consequence of the relatively small number of samples obtained at high salinity. Based on the Tokyo Bay data, with approximately 500 samples at >30 ppt, there was an apparent trend in both the proportion of samples with detectable *V. vulnificus* and the mean *V. vulnificus*/g across three temperature categories. However, even at the highest temperature category with sample information (25–30°C), mean *V. vulnificus*/g was not substantially greater than 30 MPN/g in Tokyo Bay.

For both the USA and Japanese data, mean *V. vulnificus*/g was consistently higher when salinity was <30 ppt for all temperature ranges but the difference was much more pronounced with the USA data. Part of this difference may be explained by generally higher salinities in Tokyo Bay, even when salinities are <30 ppt. For the <30 ppt salinity category, mean salinity was 24.4 ppt for the Tokyo Bay data compared with 16.8 ppt for the USA data. For the USA data, estimates of mean *V. vulnificus*/g versus the temperature categories given in Table 3 for salinities <30 ppt are consistent with the estimated regression relationship shown in Figure 2.

3.4.2. Post-harvest

The numbers of *V. vulnificus* at consumption are influenced by ambient air temperatures at harvest, the time from harvest until the oysters are placed under refrigeration, the time it takes the oysters to cool once under refrigeration, and the length of refrigeration time until consumption. Estimates of post-harvest growth addressed in this section pertain specifically to oysters harvested from moderate or low salinity regions (<30 ppt). These estimates may not be relevant to oysters harvested from high (>30 ppt) salinity areas because oysters harvested from such areas would retain high salinity levels through post-harvest transport and storage and this could substantially reduce post-harvest growth.

3.4.2.1. Growth of *V. vulnificus* from harvest to first refrigeration

The growth model used in the present assessment is the 3-phase linear growth model advocated for microbial risk assessment by Buchanan, Whiting and Damert (1997). This is the same (primary) growth model used in the FAO/WHO-VPRA and the FDA-VPRA. The growth prediction equation of this model is:

$$\log_{10}(N(t)) = \quad \min\{\log_{10}(N(0)) + \mu_m * t, A\}$$

where $N(0)$ denotes the initial number of organisms per g (i.e. at time of harvest), and $N(t)$ denotes the predicted number at t intervals of time (hours) post-harvest. The parameters of the equation are the maximum growth rate (μ_m) and the maximum density (A).

A secondary model of microbial growth relating the growth rate to ambient holding temperature is assumed. This secondary model is:

$$\mu_m(T) = \max\{0, \alpha*(T - T_0)\}$$

where T denotes hold temperature and the parameters of the equation are the temperature below which growth does not occur (T_0) and the slope (α) of a growth rate versus temperature relationship.

Comparative studies of the numbers of *V. vulnificus* in oysters received at processing plants versus oysters at harvest (Ruple and Cook, 1992) and experimental studies with shellstock oysters stored under different temperature regimes clearly indicate that post-harvest multiplication is substantial at ambient air temperatures of ~25°C, which are typical in the USA Gulf Coast in late spring through to early autumn. The best available data to estimate this parameter are provided by two studies (Cook, 1994, 1997a). It is also apparent that the minimum temperature required for growth of *V. vulnificus* is approximately 13°C (Kaspar and Tamplin, 1993). Below this temperature, *V. vulnificus* numbers decrease over time and *V. vulnificus* can enter a viable but non-culturable (VBNC) state (Oliver, 1995).

Figure 4 presents data on the growth of *V. vulnificus* in oysters where oysters were held for up to 14 hours at ambient air temperatures ranging from 24° to 32°C (Cook, 1997a). Table 5 lists the observed growth rates for *V. vulnificus* at various temperatures. A 0.75 $\log_{10}$ increase in numbers was observed over a period of 30 hours when oysters were held at 18°C (Cook, 1994). For oysters harvested during the summer and stored at ambient air temperatures ranging from 24° to 33°C (average 28°C), a 1.3 $\log_{10}$ increase in *V. vulnificus* numbers was observed over 7.5 hours, with a plateau of approximately 2 $\log_{10}$ increase after a period of 14 hours (Cook, 1997a). In an earlier study, oysters stored under refrigeration at 18°C were found to have an average increase of approximately 0.75 $\log_{10}$ over a period of 30 hours (Cook, 1994). Thus, the maximal growth rate is approximately 0.025/hour at 18°C and 0.175/hour at 28°C (for periods of less than 14 hours). Assuming no growth at 13°C, regression of maximal growth rate against temperature gives an estimate of 0.011 hr^{-1} $°C^{-1}$ for the slope factor α above the threshold temperature of 13°C (i.e. a linear regression of growth rate versus temperature above 13°C where growth rate is assumed to equal zero at 13°C). The remaining parameter in the primary growth model, the maximum density, was inferred to be equal to 10^6/g on the basis of several studies that have shown this to be the highest level found in oysters regardless of harvest and post-harvest conditions (Cook, 1994, 1997a; Cook *et al*., 2002).

Based on the parameter estimates for the primary and secondary growth models, predictions of *V. vulnificus* growth from an initial level of 3 $\log_{10}$ per g are illustrated in Figure 5 for ambient air temperatures of 18°, 20°, 26° and 32°C.

Table 5. *V. vulnificus* growth rate versus temperature**.**

Study	Holding temperature	Growth rate ($\log_{10}$ per hr)	Assumptions or limitations
Cook, 1997a	28°C	0.175	Ambient air temperature varied from 24 to 33°C, with assumed average of 28°C
Cook, 1994	18°C	0.025	Rate per hour assumed constant with observed average 0.75 $\log_{10}$ increase (n = 5) over 30-hour period
Kaspar and Tamplin, 1993	13°C		Presumed no-growth temperature

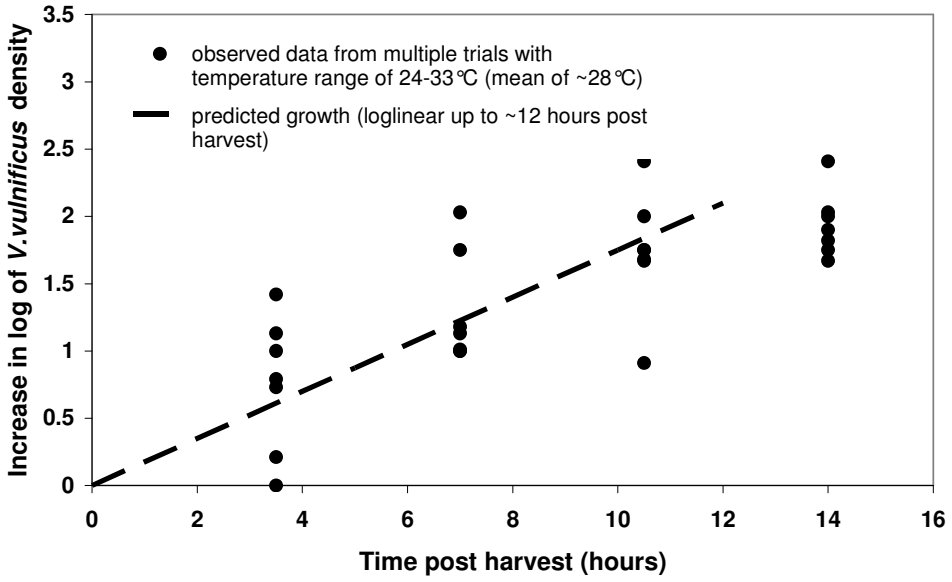

Figure 4. Post-harvest growth of *V. vulnificus*.

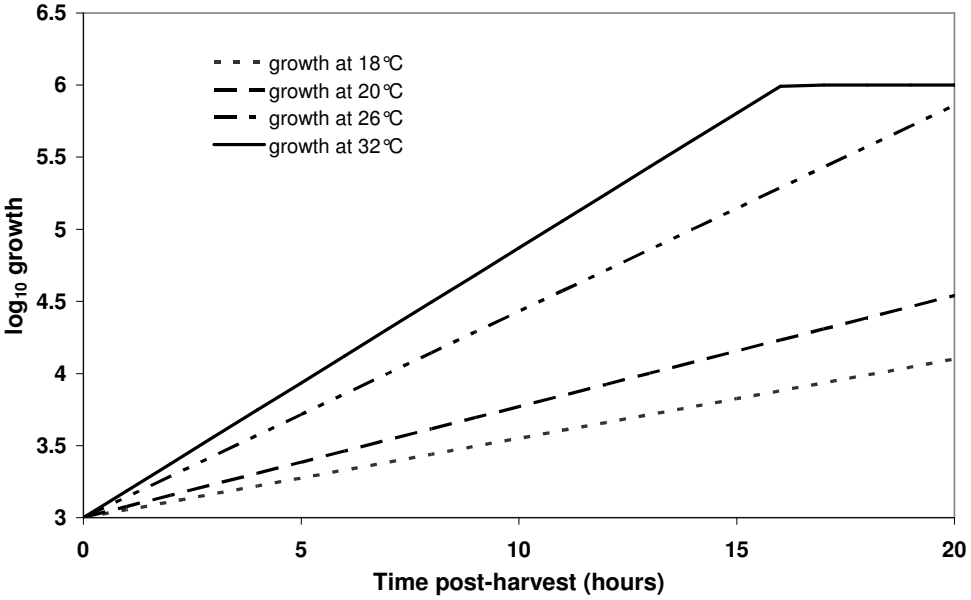

Figure 5. Predicted post-harvest growth of *V. vulnificus* from an initial level of 3 $\log_{10}$/g.

3.4.2.2. Distribution of ambient air temperature

Examination of water and air temperatures obtained from the NOAA/NBDC database (NOAA, various dates) in the USA indicate a strong correlation between water and air temperature. The air temperature in the summer is on average 1.7°C cooler than the water temperature. The standard deviation of day-to-day differences between air and water temperature is 1.3°C. The corresponding average and standard deviation in the winter are 1.1°C and 3.3°C, respectively, with air still generally cooler than water. This correlation has been incorporated into the risk simulation by modelling the distribution of the difference in water versus air temperatures based on the normal distribution within any given season. These distributions are then used to predict the air temperature that oysters would be subjected to, depending on the water temperature at the time of harvest.

Specifically, in the process of simulating the distribution of *V. vulnificus* at harvest by the Monte Carlo method, the water temperature associated with any given outcome is retained. A corresponding air temperature is obtained by first sampling from the appropriate distribution for the difference in air versus water temperature. This difference is then added to the water temperature to derive a corresponding air temperature. The distributions of differences in air versus water temperature were obtained by pooling the (seasonal) data available from a representative near-shore buoy across all available years of data. The mean and variance of these distributions are shown in Table 6.

This is the same approach to modelling of air temperatures as used in the *V. parahaemolyticus* risk assessment (FDA, 2001; FAO/WHO, in press) and was taken from that work for the purposes of this risk assessment.

3.4.2.3. Distribution of the length of time oysters are left unrefrigerated

The distribution of the length of time that oysters are held unrefrigerated can be developed by using the distribution of duration of harvesting operations (working day), with the assumption that oysters are harvested uniformly from the start of the harvest up to one hour prior to conclusion of harvesting, when they are landed and placed in cold storage. Table 7 shows the minimum, maximum and most likely duration of oyster harvesting estimated for the USA Gulf Coast. In the risk simulation, Beta-PERT distributions were used based on these parameters to simulate the variation in the duration of harvesting. A Beta-PERT distribution is a translated and scaled Beta distribution, commonly used for the purpose of simulating parameter variation in Monte Carlo simulations when only limited information is available concerning distribution (e.g. min., max., most likely value).

Table 6. Means and standard deviations of the distribution of the difference between recorded air and water temperatures (°C) at midday in the Gulf Coast of the USA.

Region (data source)	Mean (standard deviation) of the distribution of differences between air and water temperature			
	Winter (Jan-March)	Spring (April-June)	Summer (July-Sept)	Autumn (Oct-Dec)
Gulf Coast, USA (Dauphin Island, AL buoy)	-1.07 (3.3)	-1.24 (1.63)	-1.66 (1.33)	-1.62 (3.3)

SOURCE OF DATA: http://www.seaboard.nbdc.noaa.gov/Maps/Wrldmap.shtml

The parameters for these distributions were estimated from data collected during a 1995–1996 FDA Gulf Coast Seafood Laboratory (GCSL) survey in the USA (Cook, 1997b). These data included dealer-reported statistics on the length of harvest. The study was conducted in several Gulf Coast states of the USA during the autumn of two successive years; one season prior to initiation of the NSSP time-to-refrigeration requirements (for states in the USA whose product has been confirmed as the source of two or more *V. vulnificus* illnesses) and then the following year, after implementation. The survey data indicates that the state of Louisiana, with ostensibly more remote harvest areas, has substantially longer harvesting operations than the other Gulf Coast States. Distributions for time unrefrigerated were therefore developed separately for Louisiana versus the other Gulf Coast States. The overall distribution of time unrefrigerated was then obtained as the weighted recombination of these distributions, given that Louisiana accounts for ~50% of the total Gulf Coast harvest. Survey data from Texas were taken to be representative of the harvest practices of the other (non-Louisiana) states.

The duration of harvesting reported in the dealer survey data in 1996, after initiation of the NSSP requirements, was assumed to apply to the spring, summer and autumn seasons. During the winter, when cooler water conditions prevail, the temperatures are generally below the threshold associated with the shorter time-to-refrigeration requirement, so survey data obtained in 1995, prior to implementation of the NSSP, were assumed to apply.

Harvesting of oysters was assumed to occur uniformly from the start of harvest until one hour prior to the end of the harvest operation. The distribution of the duration that the oysters were held unrefrigerated was simulated by first sampling from the distribution for the duration of the harvest operation and then sampling from a uniform distribution with a minimum of one hour and a maximum corresponding to the randomly selected duration of harvest. Because they are harvested over the length of harvesting operations, the mean time that oysters remain unrefrigerated is shorter than the maximum length of duration of harvesting.

Overall, the extent of growth occurring prior to the time of first refrigeration (i.e. time at which the oysters are first placed in refrigerated storage) was simulated by:

- sampling air temperature corresponding to the water temperature at harvest;
- sampling duration of harvest;
- sampling the length of time unrefrigerated given a particular duration of harvest; and
- calculating the extent of growth expected considering both the given duration unrefrigerated and the air temperature.

As with the distribution of ambient air temperature, this is the same distribution as is used in the *V. parahaemolyticus* risk assessment and was taken from that work for the purposes of this risk assessment.

Table 7. Minimum, maximum and most likely duration of oyster harvest (length of harvesting operation) for different seasons and subregions of the USA Gulf Coast.

Location in the USA	Duration of harvest (hours)			
	Winter (Jan-March)	Spring (April-June)	Summer (July-Sept)	Autumn (Oct-Dec)
Gulf Coast - LA (50% of harvest) (pre-NSSP Control plan in LA in winter; others as ICP)	max = 13 min = 7 most likely = 12	max = 11 min = 5 most likely = 9	max = 11 min = 5 most likely = 9	max = 13 min = 7 most likely = 12
Gulf Coast - FL, AL, TX (50% of harvest) (assumed same as pre-NSSP ICP in Gulf-TX in winter; NSSP Control otherwise)	max = 11 min = 2 most likely = 8	max = 10 min = 3 most likely = 7	max = 10 min = 3 most likely = 7	max = 10 min = 3 most likely = 7

KEY TO STATES: LA = Louisiana; AL = Alabama; TX = Texas; FL = Florida.
SOURCE: Cook, 1997b.

3.4.2.4. Growth of *V. vulnificus* during cooling

The time it takes for oysters to cool once under refrigeration is assumed to vary according to the efficiency of the chilling medium, the quantity of oysters to be cooled and their arrangement in the cold room. Data on cooling rates of commercial oyster shellstock was not available. In the USA preliminary GCSL experiments with a single in-shell oyster at 30°C, in which a temperature probe was inserted into its tissue, indicated a cooling rate of approximately 0.5°C/min when placed into a 3°C cooler (A. DePaola, personal communication, 2002). However, 24 oysters in an uninsulated plastic container required approximately 7 hours to cool from 26°C to 3°C. These data suggest considerable uncertainty for cooling times after oysters are refrigerated and it was concluded that a uniform distribution between 1 and 10 hours would be appropriate to describe the current state of knowledge, with all values in this range being equally likely regardless of initial air temperature (i.e. temperature difference).

At the start of the cooling period, when oysters are first placed under refrigeration, the growth rate was taken to be equal to the initial rate as determined by ambient air temperature. At the end of the cooling period, when oysters have reached storage temperatures, it was assumed that there is no further growth and that numbers will decline slowly thereafter. Implicitly, this assumes that there is no appreciable temperature abuse after oysters have been placed in cold storage. As the rate at which oysters cool during cold storage is not known, it was assumed that during the period of cooling, the growth rate of *V. vulnificus* decreases uniformly to zero. Once again, model assumptions for growth during the process of cooling were the same as those used in the *V. parahaemolyticus* risk assessment.

3.4.2.5. Die-off of *V. vulnificus* during cold storage

V. vulnificus is more susceptible to cold than *V. parahaemolyticus*. Based on ISSC/FDA retail data (Cook et al., 2002), it has been estimated that *V. vulnificus* numbers decline by 0.041 logs per day under normal conditions of cold storage in the USA marketplace. Minimum, maximum and most likely duration of storage of oyster lots sampled in this same study were used in the FAO/WHO-VPRA to define a distribution of storage times for the USA marketplace. The same distribution was assumed here.

Data from a retail study for the time between harvest and sample collection were assumed to be a reliable estimate for the refrigerated storage time prior to consumption. Summary statistics on the storage time for samples obtained during the study are shown in Table 8. A small degree of error may be introduced by assuming that these data are representative of storage time insofar as samples were generally collected on a Monday or a Tuesday and most servings are consumed in restaurants during weekends. Since this was a year-long nationwide survey, the mean of 7.7 days and range of 1–21 days were assumed to be representative of all seasons. In the simulation, a Beta-PERT distribution based on the minimum, maximum and mode (most likely value) was used in order to obtain a smooth representation of the variation in the duration of storage time. Refrigerated storage time can vary significantly from one country to another. For example, in Japan the mean is 1 day, with a range of 0.125 to 1.04 days. In Australia it can range from 1 to 10 days, with a most likely time of 6 days, while in New Zealand, storage time tends to be shorter, between 1 and 5 days with a most likely time of 2 days (FAO/WHO, in press).

3.5. Simulation results

Monte Carlo simulations of the distribution of *V. vulnificus* at harvest and at selected points in the production-consumption process continuum were obtained using the simulation program Analytica® (Analytica, Lumina Decision Systems, Inc., USA). As outlined in the discussion above, the output distributions were based primarily on water temperature, the derived regression relationship between $\log_{10}$ *V. vulnificus* numbers and water temperature, *V. vulnificus* growth rate versus temperature and various distribution parameters, which affect the extent of microbial growth and survival post-harvest.

Seasonal *V. vulnificus* exposure associated with USA Gulf Coast oysters was simulated. Parameter distributions were obtained by the Monte Carlo method using a sample size of 10 000. The effect of year-to-year variation in mean and variance of water temperature distributions was evaluated based on 100 Monte Carlo samples of water temperature parameters (i.e. mean and variance). Using the selected Motes et al. data with the two replicates averaged, statistical summaries of the parameter distributions obtained by Monte Carlo sampling, averaged over the year-to-year variations in water temperature, are shown in Table 9.

Table 8. Summary statistics of the distribution of storage times (time under refrigeration in days) of oysters samples collected during a retail study.

Storage Time (days)	Consumed locally (within the same region of harvest)	Non-local (transported outside region of harvest)	Overall
Minimum	1	2	1
Maximum	20	21	21
Mean	6.3	9.9	7.7
Most likely	6	5	6

SOURCE OF DATA: Cook *et al.*, 2002.

Table 9. Summary output of the simulation of environmental parameters, oyster handling conditions, *V. vulnificus* growth, survival and numbers from harvest to consumption in USA Gulf Coast oysters in the winter (January - March), spring (April – June), summer (July – September) and autumn (October - December).

Distribution Parameter	Winter (January - March)	Spring (April – June)	Summer (July – September)	Autumn (October - December)
	Mean (standard deviation)[1]			
Water temperature (°C)	14.2 (2.7)	24.5 (3.5)	28.9 (1.5)	17.9 (4.5)
Log_{10} *V. vulnificus*/g at harvest	0.47 (1.09)	2.75 (0.82)	3.27 (0.64)	1.39 (1.36)
V. vulnificus/g at harvest	40	2 600	5 600	500
Air-water temperature difference (°C)	-1.07 (3.3)	-1.24 (1.63)	-1.66 (1.33)	-1.62 (3.3)
Air temperature (°C)	13.1 (4.3)	23.3 (4.1)	27.2 (2.0)	16.4 (5.5)
Time on the water	9.4 hours	7.7 hours	7.7 hours	9.1 hours
Time oysters unrefrigerated	5.2 hours	4.4 hours	4.4 hours	5.0 hours
Log_{10} growth prior to refrigeration	0.11 (0.18)	0.49 (0.32)	0.68 (0.35)	0.24 (0.30)
Log_{10} growth during cool down	0.06 (0.10)	0.37 (0.21)	0.50 (0.22)	0.15 (0.18)
Die-off during storage (in logs)	0.31	0.31	0.31	0.31
Log_{10} *V. vulnificus*/g at consumption	0.30 (1.22)	3.28 (1.08)	4.15 (0.78)	1.45 (1.64)
V. vulnificus/g at consumption	80	21 400	57 000	3 700
Oysters per serving	13.7	13.7	13.7	13.7
Grams per serving	196	196	196	196
Total *V. vulnificus* ingested per serving	1.6×10^4	4.2×10^6	1.1×10^7	7.3×10^5

NOTES: (1) For distributions that are approximately normally distributed, the standard deviation is given in parentheses; no standard deviation is tabulated for those distributions that are highly skewed. For skewed distributions (*V. vulnificus*/g), the median of the uncertainty distribution of mean *V. vulnificus*/g is given.

Overall, the simulation results suggest that *V. vulnificus* numbers increase post-harvest an average of 0.90 $\log_{10}$ MPN/g during the summer harvest season and decrease an average of 0.20 $\log_{10}$ MPN/g during the winter harvest season. Variation in water and air temperatures and the characteristics of harvesting duration and storage time have the effect of increasing the variation of *V. vulnificus* numbers at each point along the harvest-to-consumption continuum. For the USA Gulf Coast winter, the standard deviation of *V. vulnificus* numbers (in 12-oyster composites) is 1.22 $\log_{10}$ at consumption compared with 1.09 $\log_{10}$ at harvest. Due to the positive skew of the distributions, the mean density of *V. vulnificus* per gram is greater than the antilog of mean $\log_{10}$ MPN per gram. Mean densities of 57 000 and 80 per gram were obtained for the summer and winter harvest seasons, respectively. Given an average serving size of 196 grams of oyster meat weight (Cook *et al*., 2002; A. DePaola, personal communication 2002) these average numbers correspond to average ingested doses of 1.1×10^7 and 1.6×10^4 respectively.

Representative outputs of the simulation are shown in Figures 6 to 8. These graphs illustrate the effect of post-harvest parameters on the location and shape of the distribution of *V. vulnificus* per gram. Generally, each stage of the harvest to consumption continuum shifts the mean $\log_{10}$ numbers per gram, with a concomitant increase in the variability about the mean from one sample of oysters to the next.

Figure 6 shows typical distributions of the water and air temperature obtained for the Gulf Coast summer. These distributions are normal by assumption. As evident in the figure, the distribution of air temperature has a mean that is slightly less than that of water and exhibits

more variation (i.e. spread). The variation in temperatures drives numbers at harvest and determines variability in extent of growth occurring after harvest. Together with parameters affecting harvest duration (e.g. distribution of time prior to first refrigeration as shown in Figure 7), the growth rate of *V. vulnificus* post-harvest and survival during storage is used to derive the distribution of numbers at the time of consumption. The difference in *V. vulnificus* numbers at harvest versus at consumption is shown is Figure 8. The *V. vulnificus* numbers at consumption are slightly less than 1 $\log_{10}$ higher than those at harvest during the relatively higher-risk summer season.

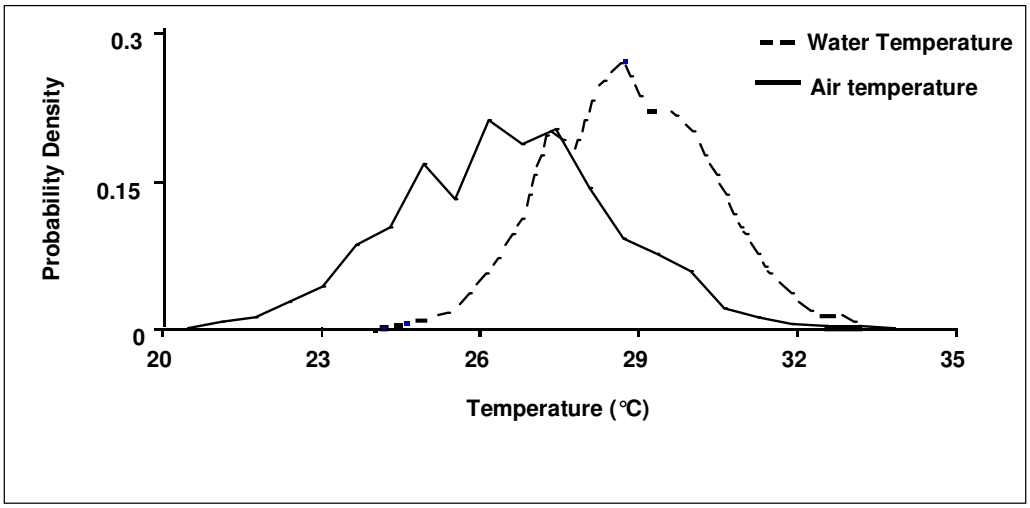

Figure 6. Distribution of typical day-to-day "noontime" water and air temperatures for the USA Gulf Coast summer.

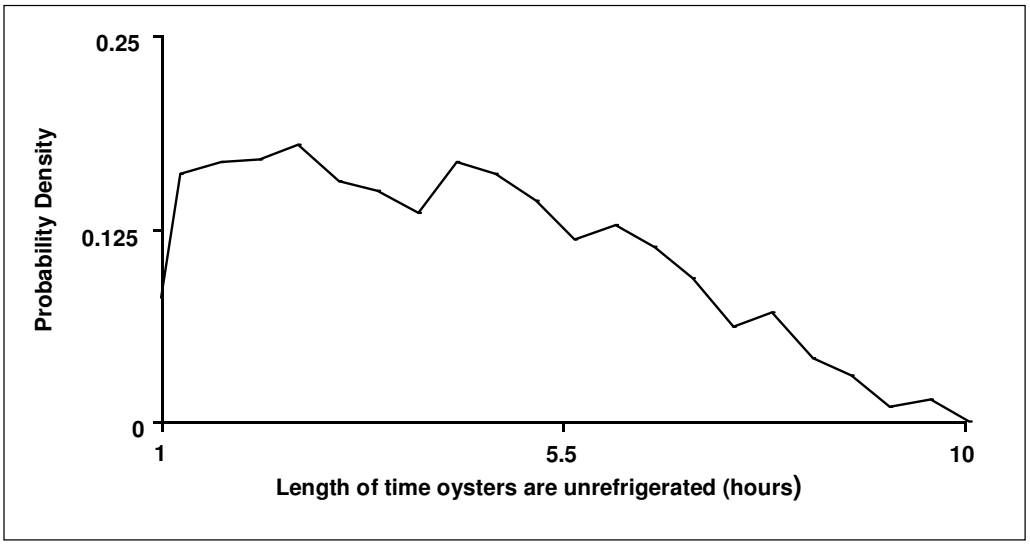

Figure 7. Distribution of the time that oysters are held unrefrigerated post-harvest.

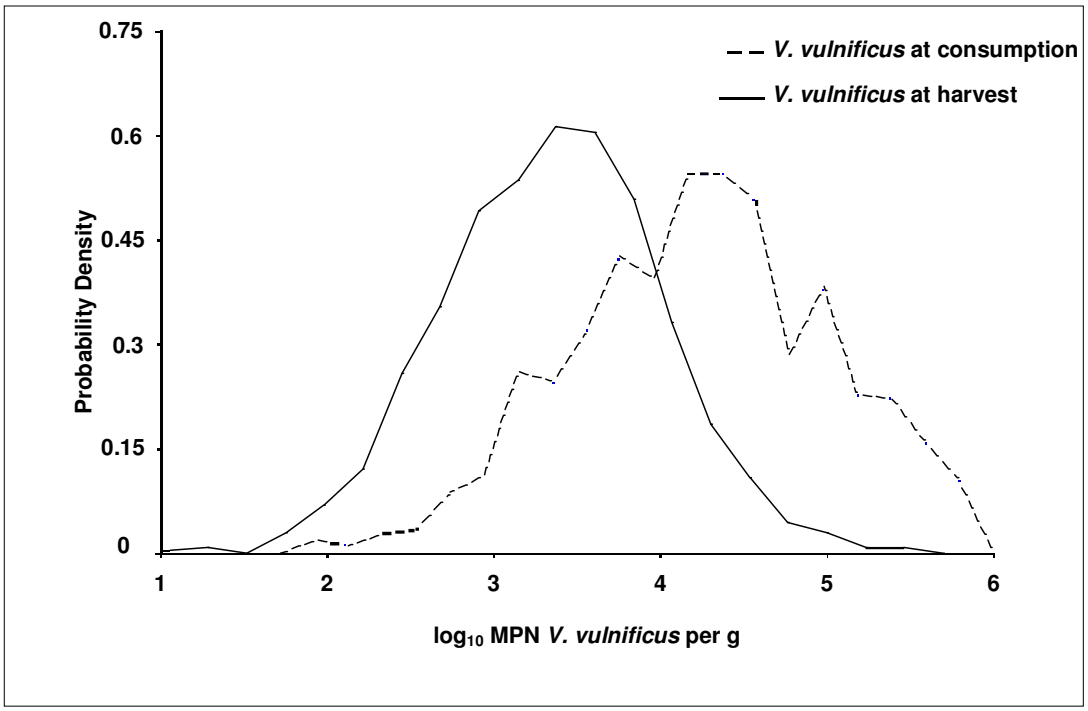

Figure 8. Distribution of *V. vulnificus* (Vv) numbers at harvest versus consumption.

3.6. Model validation

Results of simulations were compared with available data on the density of *V. vulnificus* in oysters at retail in the USA. Summary statistics for the density of *V. vulnificus* in oysters at retail, obtained by the ISSC/FDA collaborative retail study (Cook et al., 2002), are presented in Table 10. The model simulation output based on the Motes et al. data with the two replicates averaged is generally consistent with these measurements, but there are noticeable discrepancies. Mean *V. vulnificus* per gram is over-predicted during some seasons and under-predicted during others. Similarly, mean $\log_{10}$ *V. vulnificus* per gram, which approximates the median of the distribution of *V. vulnificus* per gram, is substantially under-predicted in the autumn and to a lesser degree in the winter season, but not during the spring and summer seasons (Figure 9).

Table 10. ISSC/FDA retail data on the numbers of *V. vulnificus* in USA Gulf Coast oysters.

Region and season in the USA	No. of samples	Samples with detectable *V. vulnificus*	*V. vulnificus*/g[(1)]	Log_{10} *V. vulnificus*/g[(2)]
Gulf/winter	37	84%	60	0.60 (1.12)
Gulf/spring	46	96%	40 800	3.24 (1.28)
Gulf/summer	41	97.5%	62 100	4.00 (1.16)
Gulf/autumn	46	96%	10 700	3.08 (1.48)

NOTE: (1) Arithmetic mean. (2) Mean and standard deviation.

The differences between observed versus predicted mean $\log_{10}$ *V. vulnificus* per gram appear to be largely a consequence of warmer than normal water (and air) temperatures that were occurring in the USA Gulf Coast from September 1998 to March 1999 due to a La Nina weather pattern. As will be discussed further in later sections of this document, substituting the autumn-1998-specific and winter-1999-specific temperature data in the simulation reduces much of the discrepancy in predicted versus observed mean $\log_{10}$ *V. vulnificus* per gram (Figure 9). Differences in observed versus predicted mean *V. vulnificus* per gram are more problematic. To the extent that *V. vulnificus* per gram is approximately lognormal, the mean of the distribution of *V. vulnificus* per gram is a function of both the mean and standard deviation of the distribution of $\log_{10}$ *V. vulnificus* per gram. Thus, over-prediction of mean *V. vulnificus* per gram by the model simulation could be due to over-prediction of either the mean or the standard deviation of $\log_{10}$ *V. vulnificus* per gram.

Since, after correcting for temperature, differences in predicted versus observed mean $\log_{10}$ *V. vulnificus* per gram are not large (Figure 9), it appears more likely that differences in observed versus predicted mean *V. vulnificus* per gram are attributable to over-prediction of the population standard deviation of $\log_{10}$ *V. vulnificus* per gram. Here it is relevant to note that the standard deviation of observed $\log_{10}$ *V. vulnificus* per gram shown in Table 10 is likely to be inflated by the presence of method error.

The ISSC/FDA retail study (Cook et al., 2002) employed an MPN procedure for enumeration and thus a percentage of the variance of observed $\log_{10}$ numbers is attributable to method error over and above true variation in numbers from one sample to the next. This method error is symmetric and unbiased on the $\log_{10}$ scale but not on the untransformed scale (i.e. MPN or count per gram). The magnitude of this method error is not known precisely since a modified MPN procedure was employed to compensate for the effect of interference observed in the MPN series for these data. Thus, method error could not be estimated and its effect subtracted from the estimates shown in Table 10. The effect of not correcting the estimates for the presence of this method error introduces an upward bias in the estimates of mean *V. vulnificus* per gram. In the model simulation, the error associated with measurements of *V. vulnificus* has already been subtracted out and, consequently, the over-prediction of mean *V. vulnificus* per gram in some seasons may be even greater than that suggested by a simple comparison of the numbers shown in Tables 9 and 10.

Table 11 compares mean *V. vulnificus*/g observed in the ISSC/FDA (Cook et al., 2002) retail study with predicted values at consumption using both average seasonal temperatures and temperature specific to the autumn of 1998 and the winter of 1999 based on the Motes et al. (replicates averaged) and Tamplin data alone and with the pooled data sets. The highest predicted *V. vulnificus* levels for all seasons were obtained based on the Tamplin data and the lowest with the Motes et al. data. The *V. vulnificus* levels observed in the ISSC retail study were considerably lower than any of the predicted values for the autumn of 1998 and the winter of 1999; the Tamplin data over-predicted by a factor of approximately 3 and 20 for the autumn and winter, respectively. Overall, the predictions based on the Motes et al. data were in closest agreement with the retail data but it under-predicted in the spring by a factor of ~2 and over-predicted in the winter by a factor of ~6.5. The estimate of population standard deviation at time of harvest is particularly important, since this is the largest component of the variance in $\log_{10}$ *V. vulnificus* levels at the time of consumption. It is possible that the estimates obtained from analysis of the Motes et al. and Tamplin data are overestimates of the true variation. As discussed in Appendix B and D, there was substantially higher variance in *V. vulnificus* levels observed in the Tamplin study compared with that observed in the Motes

et al. study. This may be a consequence of the fact that the studies were undertaken in different periods, locations or that different protocols were used to ensure that no post-harvest growth occurred in collected samples. It is also possible that the true variation in *V. vulnificus* levels changes with season and this is not reflected in the parameter estimates that were obtained from the regression analysis of the harvest density data.

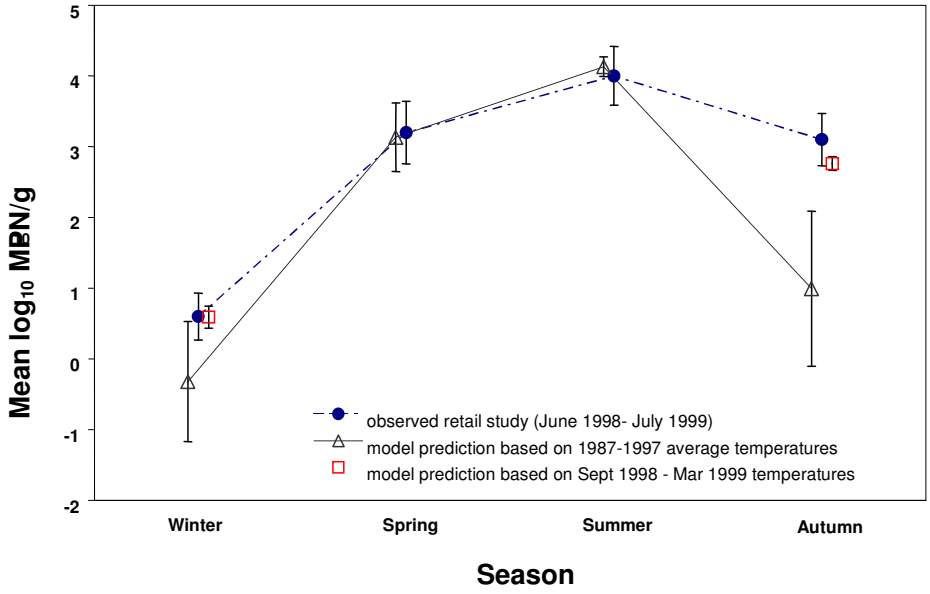

Figure 9. Predicted versus observed *V. vulnificus* numbers in retail shell oysters. The error bars denote the 95% confidence intervals for mean $\log_{10}$ levels with respect to the retail data and corresponding uncertainty intervals of the predictions based on model simulations. Best estimates of model-based predictions were taken to be equal to the medians of the respective uncertainty distributions.

Table 11. ISSC/FDA retail data (Cook et al., 2002) on the numbers of *V. vulnificus* in USA Gulf Coast oysters compared with alternative model-predicted numbers at consumption derived from different data sets for levels at harvest.

Region/ Season in USA	Mean *V. vulnificus*/g based on retail data	Mean *V. vulnificus*/g (based on Motes data model)(1)	Mean *V. vulnificus*/g (based on Tamplin data model)(1)	Mean *V. vulnificus*/g (based on pooled data model)(1)
Gulf/winter (typical)	–	80 (20, 610)	240 (30, 1 800)	140 (20, 1 000)
Gulf/spring (typical)	40 800	21 400 (12 700, 33 500)	55 300 (21 800, 92 100)	36 600 (24 100, 55 800)
Gulf/summer (typical)	62 100	57 000 (37 900, 70 500)	131 800 (63 100, 203 000)	93 400 (72 900, 107 000)
Gulf/autumn (typical)	–	3 700 (1 500, 11 700)	9 200 (3 300, 25 600)	6 100 (2 700, 16 500)
Gulf/autumn 1998	10 700	16 100 (11 800, 19 800)	36 600 (16 600, 59 800)	25 400 (21 100, 28 800)
Gulf/winter 1999	60	390 (240, 590)	1 100 (400, 2 200)	670 (420, 1 000)

NOTES: (1) median of the uncertainty distribution of model-based simulations and the central 90% interval of the uncertainty distribution (5 and 95 percentiles).

4. Hazard characterization

4.1. Description of the factors that influence the disease outcome

4.1.1. Characteristics of the pathogen

V. vulnificus potentially causes mild to moderate gastroenteritis in healthy people who consume contaminated food; however, for a specific subpopulation of susceptible people, *V. vulnificus* can cause a serious septicaemia that frequently leads to death.

4.1.1.1. Genetic factors, such as virulence factors

The virulence factors associated with *V. vulnificus* are poorly characterized. Factors like cytolysin, protease/elastase and phospholipase all may play a role, but none appears to be essential for virulence, as some mutant strains with these factors deleted do not appear to exhibit decreased virulence in animal models (Strom and Paranjpye, 2000). The presence of a capsule appears to be correlated with virulence, but most freshly isolated environmental strains appear to have a capsule, irrespective of their virulence (Strom and Paranjpye, 2000). The virulence factors associated with *V. vulnificus* include a capsule, cytolysin, protease/elastase and phospholipase, but these are found in nearly all clinical and environmental strains (Strom and Paranjpye, 2000). More recent studies (Nilsson et al., 2003) indicate that an rRNA gene sequence may be related to virulence, as the type B sequence was more prevalent in clinical strains (approximately 80%) than in strains isolated from market oysters (approximately 8%). While rRNA type may be a potential virulence marker, the seasonal and regional distribution of rRNA type has not been investigated. Virulence appears to be multifaceted and is not well understood, and therefore, in this risk assessment, all strains were considered virulent.

4.1.2. Characteristics of the host

4.1.2.1. Immune and physiological status of the host

Foodborne *V. vulnificus* infection is clearly associated with underlying medical conditions (Strom and Paranjpye, 2000). Liver disease is a prominent risk factor for *V. vulnificus* infection, including cirrhosis due to alcohol consumption. Additional risk factors include diabetes, gastrointestinal disorders (surgery, ulcers), haematological conditions, and immunodeficiency due to underlying conditions such as cancer and treatment of chronic conditions with immunosuppressive agents (arthritis, etc.). As with many other micro-organisms, the pathogenicity of *V. vulnificus* appears to be associated with the availability of free iron in the host (Wright et al., 1981). Many of the known predisposing conditions for infection, particularly chronic liver diseases, are associated with impaired iron metabolism. *V. vulnificus* may pose a small risk to otherwise "healthy" individuals since a small fraction of cases (<5%) occur in individuals without any identifiable risk factor.

Normal population

The normal population may be susceptible to a relatively mild gastroenteritis from consumption of seafood's harbouring *V. vulnificus*, but this rarely leads to primary septicaemia (Strom and Paranjpye, 2000). Hence, healthy individuals will be excluded from this assessment, as the focus is the more serious primary septicaemia cases.

Susceptible population

The prevalence of predisposing conditions among the adult population (>18 years of age) in the USA has been estimated in a 1997 memorandum to the FDA Office of Seafood Director (Klontz, 1997). These estimates are presented in Table 12.

Using a median value for the prevalence of hepatitis and lupus, these numbers suggest that approximately 7% of the USA adult population is susceptible to infection. Given the uncertainty in prevalence of liver disease (including hepatitis), this number could be as high as ~16%. However, the median estimate of prevalence of liver disease is consistent with results of the 1988 Florida behavioural survey in which 2.4% of raw oyster consumers surveyed reported that they were aware that they had a liver disease (Hlady, Mullen and Hopkin, 1993). Consequently, although it can not be ruled out that up to ~8% of the population have undiagnosed chronic liver conditions, a figure of 7% appears to be a more reasonable estimate of the susceptible population. This represents a population of 13 million individuals in the USA at "high risk" of infection. However, it must be noted that this could be different for other countries and regions, particularly where large numbers of the population suffer from hepatitis.

The overall estimate of size of the susceptible population is somewhat imprecise due to varying case definitions of the disease conditions. For most disease conditions, the estimates presented in Table 12 (Klontz, 1997) are based on cases defined by relatively severe progression (e.g. long-term corticosteroid treatment, end-stage renal disease, etc.). The

Table 12. Prevalence rates of *V. vulnificus* risk factors per 100 000 individuals assuming a total USA adult population of 185 000 000 individuals (Klontz, 1997).

Risk factor	Prevalence per 100 000 individuals
Diabetes (insulin-dependent)	540.5
Liver disease (cirrhosis)	2000 (range: 1600–9900)
Gastric acidity	38.9
Cancer	1420.0
Hepatitis (B and C)	(range: 400–1600)
Kidney disease	108.0
Haemochromatosis	1081.1
AIDS	540.5
Immune-compromised due to treatment/surgery	
Asthma	25.7
Rheumatoid arthritis	51.4
Psoriatic arthritis	37.9
Lupus	(range: 4–250)
Polymylagia rheumatica	53.0
Giant cell arthritis	12.0
Transplant recipients	59.5
Total	~7000 (7%)

relative risks of infection associated with the identified disease conditions have not been well characterized. There may be a distribution of susceptibility related to progression of each of the various predisposing conditions. For this assessment, relative risks have been assumed to be the same across these identified disease conditions, namely all susceptible individuals are equally susceptible.

4.1.2.2. Age, sex and ethnic group

The vehicle of infection under consideration in this risk assessment for *V. vulnificus* is raw oysters. The consumption patterns for raw oysters in the USA have been estimated for age, sex and ethnic group (Desenclos *et al*., 1991; Klontz et al., 1995; Timbo *et al*., 1996; and Flattery and Bashin, 2004). Women comprise only about 10% of reported *V. vulnificus* infections (M. Glatzer, personal communication, 2001). While women consume raw oysters less frequently than men (Flattery and Bashin, 2004), this does not account for the reported magnitude of differences in illness rates. It is not known whether women who consume oysters have different rates of risk factors or some additional protection compared with men.

4.1.2.3. Health behaviours

All *Vibrio* spp. are relatively susceptible to inactivation by cooking. Most of the risk associated with the relevant strains of *Vibrio* spp. in food comes from the consumption of raw oysters or from cross-contamination of other foods by raw seafood or contaminated water. Health behaviours leading to impaired liver function as the result of long-term heavy alcohol consumption are a major risk factor for septicaemia from *V. vulnificus* infection (Klontz, 1997).

4.1.2.4. Genetic factors

Host genetic factors related to susceptibility to *V. vulnificus* gastrointestinal infections are unknown, however, there are many genetic factors associated with the likelihood of the infection to proceed to septicaemia. The presence of a human genetic mutation leading to reduced levels of transferrin, such as hereditary haemochromatosis, results in increased likelihood of septicaemia for the infected individual.

4.1.3. Characteristics of the food matrix

4.1.3.1. Fat and salt content

Fat and salt content are probably not relevant in the determination of risk with respect to *Vibrio* spp. While the fat content of a matrix may be relevant with respect to the increase of effective dose of pathogens through protection of *Vibrio* spp. in micelles during gastric passage, there is insufficient evidence to model the degree of increased survival.

4.1.3.2. pH and water activity

Vibrio spp. appear to be relatively sensitive to both low pH and dehydration. Because of the nature of most foods associated with the unintended consumption of *Vibrio* spp., pH and water activity are probably not relevant in modelling survival of *V. vulnificus* in raw oysters, although these parameters may be relevant in modelling the growth of *Vibrio* spp. in other foods as a result of cross-contamination.

4.2. Public health outcomes

There are numerous reports of sporadic foodborne cases of illness caused by *V. vulnificus,* but outbreaks of illness due to *V. vulnificus* have not to date been associated with consumption of food (Shapiro et al., 1998). An outbreak of wound infections caused by a single clone, designated biogroup 3, was reported among fish handlers in Israel, but there have not been subsequent reports of outbreaks (Bisharat and Raz, 1997).

4.2.1. Manifestations of disease

V. vulnificus causes mild to severe gastrointestinal illness, potentially progressing to septicaemia, with a significant mortality rate among susceptible populations.

4.2.2. Rationale for the biological end points modelled

Septicaemia was the endpoint modelled in this risk assessment, as patients typically present to healthcare systems with this symptom. Based on the available data, this risk assessment considers only reported cases in which a history of consumption of raw Gulf Coast oysters is documented. Because of the severity of the septicaemia, under-reporting was not considered to be substantial (2:1) compared to gastrointestinal illnesses, such as is caused by *V. parahaemolyticus*, which the Centers for Disease Control (CDC) estimates to have a 20:1 under-reporting ratio in the USA (Mead et al., 1999). Additional sources of under-reporting of *V. vulnificus* septicaemia have been suggested, e.g. non routine use of Thiosulfate Citrate Bile Sucrose (TCBS) agar in analysis of samples from non-hospitalized patients (J. Painter, personal communication, 2004).

Data collected by FDA was used in this assessment. Historically, FDA has recorded cases only where patients admitted eating oysters. Patients who ate oysters may have denied oyster consumption, may not have been willing to answer questions, or may have deceased before a food history could be obtained. Alternatively, the number of oyster-associated infections could be estimated from those that did respond to the questionnaire. Another source of under-reporting is the failure to capture all the cases in different reporting systems. Since 1998, most state health departments voluntarily report *V. vulnificus* infections to the CDC Cholera and other Vibrios Surveillance System (COVISS). COVISS is the source of case reports referenced in this risk assessment. However, states may also choose to report to the National Notifiable Diseases Surveillance System (NNDSS). In a recent comparison of both surveillance systems, CDC found that approximately 80% of *V. vulnificus* cases were in common and approximately 20% of cases reported to one system were not reported to the other. Applying the capture-recapture method indicated that multiplying the total number of reported cases to COVISS by 1.25 (1/80%) would be appropriate for estimating the total number of reported cases. Applying the above adjustments would indicate that there are 2.5 times more *V. vulnificus* septicaemia cases associated with raw Gulf Coast oysters than the mean of 32 reported cases from 1995 to 2001. It is possible to make adjustments to account for any possible under-reporting. Although no such adjustments were undertaken in the current risk assessment, it involves shifting the dose-response relationship toward greater risk at a given dose which in turn would lead to an increase in the illness reduction predictions from interventions by a factor of 2.5. For example, a mitigation targeted at reducing *V. vulnificus* to one reported case per year based on this risk assessment, would if adjusted for under-reporting compare to 2.5 annual cases: however while the current risk assessment

predicts the number of illnesses prevented to be 31, adjusting for under-reporting would lead to a prediction of 77.5 (31× 2.5) illnesses prevented.

4.3. Dose-response relationship

4.3.1. Summary of available data

4.3.1.1. Probability of illness given exposure.

There are no human volunteer studies using *V. vulnificus* with which to estimate a dose-response relationship. The available data allows estimation of the relationship by comparing monthly exposure estimates for sensitive populations with monthly observed epidemiological data in the USA in a manner similar to that proposed by Buchanan et al. (1997). A consistent *V. vulnificus* reporting system has been in effect since 1995 in the USA, and CDC data is currently available up to and including 2001.

4.3.1.2. Probability of sequelae given illness and secondary and tertiary transmission

There are no known sequelae associated with *V. vulnificus* septicaemia. There are few, if any, reports of secondary or tertiary transmissions of illnesses caused by *V. vulnificus*.

4.3.1.3. Probability of death given illness

For *V. vulnificus* in the USA, mortality rates are between 50% and 60% for patients with septicaemia (Hlady and Klontz, 1996; Shapiro *et al*., 1998).

4.3.2. Sources of data used

Modelling of the dose-response relationship for *V. vulnificus* was based on estimates of exposure per eating occasion, number of eating occasions in the susceptible population, and month- and year-specific data on the number of oyster-associated cases reported to the USA CDC from 1995 to 2001, as shown in Appendix C. Both the Beta-Poisson and the Exponential model were fitted to the data and the uncertainty of the Beta-Poisson dose-response fit was characterized by considering uncertainty or variability in the number of cases likely to occur at any given exposure and the uncertainty of the mean $\log_{10}$ density of *V. vulnificus* at harvest predicted to be associated with month- and year-specific water temperatures in the Gulf of Mexico.

Typical calculations of mean *V. vulnificus* dose per serving and USA CDC statistics for average number of cases per month are shown in Table 13. The mean *V. vulnificus* numbers in oyster tissue at harvest were obtained by combining data for the USA Gulf Coast water temperatures with the *V. vulnificus* density versus water temperature regression relationship presented in the exposure assessment.

The mean *V. vulnificus* levels at retail were developed in the exposure assessment based on post-harvest handling assumptions, as reported in Section 3, together with estimates of *V. vulnificus*-specific growth rate post-harvest and survival during cold storage. The same methods outlined in the exposure assessment were used here to estimate monthly mean *V. vulnificus* densities at retail. While the estimates shown in Table 13 are typical averages, examination of the water temperature and case series data suggested the potential for year-to-

year differences in water temperature affecting illness rates. Consequently, month- and year-specific data were used to develop the dose-response assessment, and the numbers shown in Table 13 are simply intended to be illustrative of the type of calculations that were employed.

The number of servings per month was estimated assuming that 50% of the average landings for that month are consumed raw and then converting to the corresponding number of meals based on average oyster weight and typical number of oysters per serving. While there were some year-to-year differences in aggregate monthly oyster landings, these differences were not substantial compared with the potential effects of water temperature. Consequently, average monthly landing statistics, as shown in Table 13, were used for all years (1995–2001). The number of meals consumed by the susceptible population was estimated as being 7% of total meals. For the purpose of dose-response modelling, the average ingested *V. vulnificus* dose per serving was considered more pertinent than the median (Crump, 1998). The mean is the more pertinent summary statistic in this context as it has been observed that it is the illness burden that is the public health impact of foremost concern and this is in turn determined by the mean (and not necessarily the median) risk per serving in respect to any given collection of (variable) exposures for which illness burden is of interest (see Appendix C). Thus an estimated mean *V. vulnificus* dose per serving was matched with each month- and year-specific number of reported cases, and the modelled relationship was between mean risk and mean dose rather than individual-level risk versus individual-level dose (see Appendix C). The numbers of reported month- and year-specific cases of septicaemia used in this dose-response assessment were those occurring and reported to CDC between 1995 and 2001 (M. Glatzer, personal communication, 2002). Reported cases prior to 1995 were not used since the extent of under-reporting may have been more substantial prior to 1995.

Based upon the estimated doses and epidemiological data, Beta-Poisson and Exponential curve fits were obtained by maximum likelihood. Since the number of servings consumed is large relative to the number of illnesses reported in any given month and year, a Poisson regression approach was used as an appropriate approximation to a Binomial regression. The Exponential model, although not statistically rejected, did not otherwise provide a satisfactory fit to the data and was therefore not considered further with respect to risk characterization (see Appendix C).

An uncertainty analysis of the Beta-Poisson fit was obtained by generating 100 alternative data sets representing uncertainty or variability in the number of cases that could potentially occur in a given month and year and the uncertainty of estimated month- and year-specific mean *V. vulnificus* numbers at harvest. The exposure assessment utilized a quadratic regression to predict *V. vulnificus* numbers versus temperature. The asymptotic standard errors and correlations between the parameter estimates in this regression model were used to define a multinormal uncertainty distribution for the parameters determining mean $\log_{10}$ *V. vulnificus* numbers versus water temperature, and the residual variation of $\log_{10}$ *V. vulnificus* numbers independent of temperature.

Monte Carlo samples from this distribution were used to generate alternative sets of month- and year-specific mean ingested dose by applying the same harvest-to-consumption calculations discussed above. The effect of uncertainty in growth and survival rates post-harvest was not considered. In consideration of potential uncertainties in case reporting and the variability in number of cases that could occur under identical exposure conditions, the uncertainty of month- and year-specific risk estimates was characterized by varying month-

Table 13. Estimated monthly mean *V. vulnificus* per gram and *V. vulnificus* per serving at time of consumption based on environmental (water temperature) data versus average USA case burden based on epidemiological reporting data for *V. vulnificus*.

Month	Mean and std deviation of water temperature (°C)[1]	Mean $\log_{10}$ *V. vulnificus*/g		Landings (kg)[2]	Total meals	Servings for at-risk individuals	*V. vulnificus*/serving (dose)		Average no. of Cases[3]
		At harvest	At consumption				Median	Mean	
Jan	12.9 (2.9)	0.06	-0.11	719 398	1 835 000	128 000	155	14 000	0.14
Feb	15.1 (2.8)	0.73	0.65	737 541	1 882 000	132 000	890	70 000	0.14
Mar	17.4 (2.0)	1.41	1.47	847 311	2 162 000	151 000	6 000	109 000	0.29
Apr	21.7 (1.7)	2.36	2.68	733 459	1 871 000	131 000	94 900	675 000	1.86
May	25.8 (1.9)	2.98	3.65	616 432	1 573 000	110 000	888 000	5 025 000	4.57
Jun	28.8 (1.4)	3.26	4.15	588 309	1 501 000	105 000	2 855 000	11 561 000	3.14
Jul	30.0 (1.2)	3.32	4.31	542 043	1 383 000	97 000	4 095 000	15 598 000	4.14
Aug	30.3 (1.0)	3.33	4.33	492 148	1 256 000	88 000	4 231 000	16 536 000	5.14
Sep	28.2 (1.7)	3.21	4.02	555 197	1 416 000	99 000	2 074 000	9 008 000	4.71
Oct	22.7 (2.7)	2.51	2.94	710 779	1 813 000	127 000	173 000	1 943 000	4.43
Nov	18.4 (2.8)	1.64	1.71	817 827	2 086 000	146 000	10 300	257 000	2.71
Dec	15.4 (2.5)	0.84	0.77	835 064	2 130 000	149 000	1 170	39 000	0.71

NOTES: (1) Average monthly water temperatures at Dauphin Island and Weeks Bay, Alabama, USA, 1995–2001. (2) Average 1990–1998. (3) Based on confirmed illnesses 1995–2000.

and year-specific case incidence as a Poisson random variable with mean equal to the month- and year-specific case incidence reported to the CDC.

Figures 10 and 11 show the maximum likelihood estimate of the Beta-Poisson dose-response curve for *V. vulnificus* septicaemia. The dots represent the best estimates of the month- and year-specific exposure based on water temperatures and the risk of illness based on the observed epidemiology (1995–2001). The solid line is the most likely Beta-Poisson model and the parallel dashed lines are 90% upper and lower uncertainty limits on the predicted risk based on the uncertainty factors identified and considered here. Figure 10, which illustrates the relationship between log risk and log dose, excludes instances for which the reported number of month- and year-specific cases was zero. However, estimated data points with zero reported cases still influence the maximum likelihood fit of the Beta-Poisson curve, as indicated by the fact that the curve and confidence interval are substantially below the data points plotted for exposures less that 10^5.

Figure 11 showing the relationship between risk and $\log_{10}$ dose indicates the influence of the estimated data points for which the reported number of cases was zero. The best estimates of the parameters for the Beta-Poisson model are $\alpha = 9.3\times10^{-6}$ and $\beta = 110\,000$. A Monte Carlo uncertainty sample of the alpha and beta parameters of the Beta-Poisson model resulting from the identified uncertainties of exposure and risk is shown in Figure 12. As is evident from this sample, the confidence region for $\log(\alpha)$ and $\log(\beta)$ is an ellipsoid with strong correlation between uncertainty of $\log(\alpha)$ and $\log(\beta)$.

4.3.3. Assumptions

In developing the dose-response model for *V. vulnificus*, the following assumptions were made:

- the mean meal size is 196 g per serving;
- seven percent of the population is at risk (Klontz, 1997) and this population consumes raw oysters with the same frequency as non-susceptible individuals;
- the estimated mean number of *V. vulnificus* cells per gram at consumption is based on a regression equation for $\log_{10}$ *V. vulnificus*/g versus temperature at harvest, average time unrefrigerated, estimated growth rate versus temperature, and survival in cold storage;
- the estimates of monthly mean *V. vulnificus* per serving, based on the exposure analysis conducted, are assumed accurate. These estimates are based on the assumption that no temperature abuse occurs after oysters reach no-growth temperatures; and
- the use of mean *V. vulnificus*/g rather than median *V. vulnificus*/g (i.e. mean $\log_{10}$ *V. vulnificus*/g) as a summary measure of exposure for a group is considered appropriate for dose-response analysis (i.e. for grouped data with varying individual doses within each group it is considered more appropriate to relate the average response to average (mean) dose).

4.3.4. Goodness of fit

The goodness of fit of the dose-response models considered (Beta-Poisson and Exponential) was assessed based on the Deviance (McCullagh and Nelder, 1989), which is a likelihood ratio statistic contrasting the maximum likelihood attained under a specified model compared with the maximum possible likelihood, without any constraint. Although both dose-response

models were found to provide adequate fit statistically, the fit of the Exponential model was found to be appreciably worse than that of the Beta-Poisson (see Appendix C).

4.3.5. Uncertainty and variability

This analysis incorporates both uncertainty and variability in the estimates.

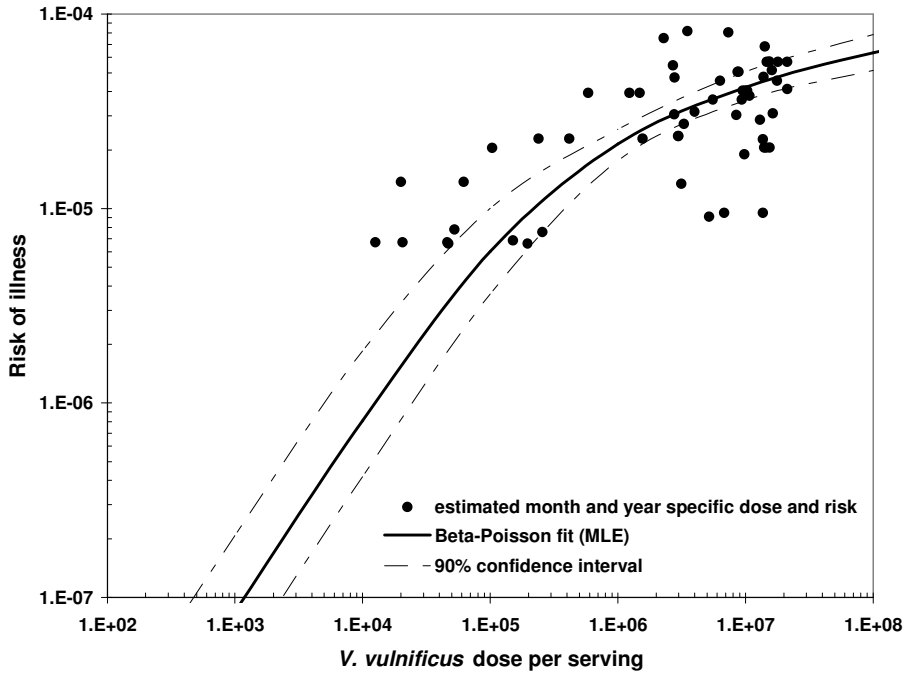

Figure 10. Log-log plot of Beta-Poisson dose-response curve for *V. vulnificus* ($\log_{10}$ of monthly average risk per serving versus $\log_{10}$ of monthly average dose per serving). Each point is determined by the risk of reported oyster-related illness (number of observed cases divided by the estimated number of servings) and the mean exposure corresponding to month- and year-specific water temperature data.

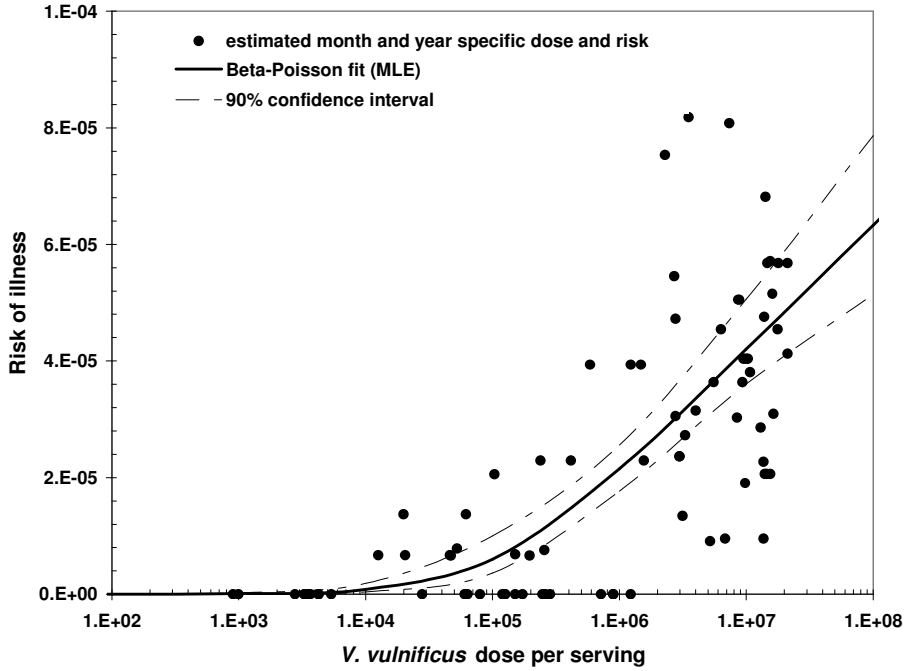

Figure 11. Semi-log plot of Beta-Poisson dose-response curve for *V. vulnificus* (monthly average risk per serving versus $\log_{10}$ of monthly average dose per serving). Each data point is determined by the risk of reported oyster-related illness (number of observed cases divided by the estimated number of servings) and the mean exposure corresponding to month- and year-specific water temperature data.

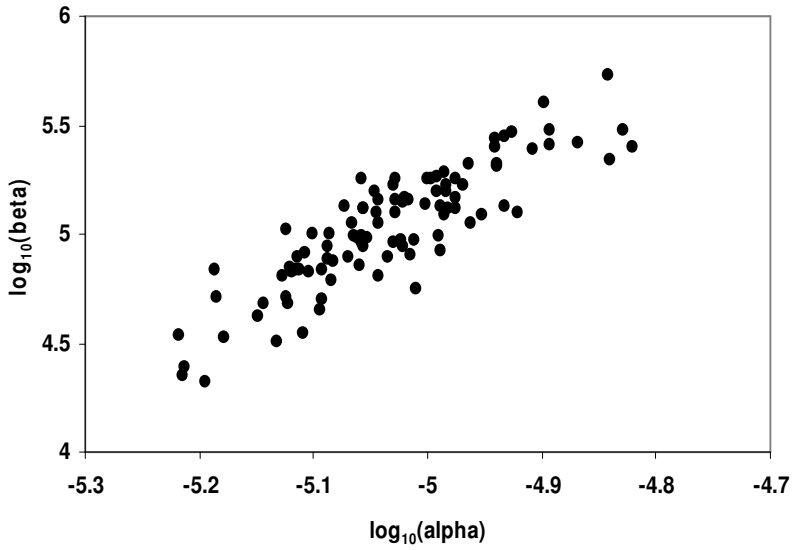

Figure 12. Parameter uncertainty of the Beta-Poisson dose-response model fit to *V. vulnificus* epidemiological data and estimated month- and year-specific mean exposure.