Draft Risk Assessment on the Public Health Impact of *Vibrio parahaemolyticus* in Raw Molluscan Shellfish

Center for Food Safety and Applied Nutrition
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Prepared by the *Vibrio parahaemolyticus*
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COMMENTS AND INFORMATION REQUESTED DURING PUBLIC COMMENT PERIOD

The Food and Drug Administration has always committed itself to bringing the best science available to all of its decisions and activities, which is the underlying reason that this and other quantitative microbial risk assessments are undertaken. As with any risk assessment, the knowledge available for the conduct of the current risk assessment was incomplete and assumptions had to be made when specific data were lacking or limited in scope. When such assumptions were made, we have attempted to seek the best scientific information available including having all assumptions and modeling approaches reviewed by the National Advisory Committee on Microbiological Criteria for Foods and the interagency Risk Assessment Consortium. However, to ensure that we have both identified all key data sources and submitted the assessment to a rigorous peer review, we are releasing the assessment in draft form. A comment period has been established during which we will be actively seeking comments, suggestions, and additional data sources. Written comments should be submitted to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852 within 60 days after publication of the document. Two copies of comments are to be submitted, except that individuals may submit one copy. Comments must be identified with the Federal Register Docket No. 99N-1075. Some of the key areas that we are seeking comments are listed below. The information acquired during the comment period will be reviewed and used, as appropriate, to further enhance the risk assessment and decrease uncertainty to the greatest degree possible. As stated in the Federal Register, Docket No. 99N-1075, the preliminary results of the draft *V. parahaemolyticus* risk assessment will be presented at a public meeting during the comment period.

Examples of specific assumptions made in the risk assessment for which we are seeking comments and additional data include:

- Pathogenesis was based on the presence of the most characterized virulence factor of the organism, thermostable direct hemolysin (TDH). Based on the levels from the literature, levels of pathogenic *V. parahaemolyticus* used ranged from 1.6% to 6.4% for the West Coast; 0.1% to 0.4% remaining regions.

- Time after harvest to refrigeration was based on a 1997 GCSL survey that included dealer reported statistics on the length of harvest *(28)*, as well as on the NSSP time-temperature control plan.

- All *V. parahaemolyticus* in oysters, regardless of pathogenicity, have similar growth and survival rates.

- The growth rate of *V. parahaemolyticus* in oysters is approximately 1/4 that of the growth rate in broth at all environmental temperatures.

- Lag time to growth of *V. parahaemolyticus* in oyster after harvest is negligible.

- The growth rate of *V. parahaemolyticus* drops uniformly down to zero during the period of initial refrigeration following harvest.

- Depuration is ineffective against *V. parahaemolyticus* in oysters.

- Equal consumption patterns were assumed between immune compromised and healthy populations.

- Equal virulence was assumed for all pathogenic *V. parahaemolyticus.*

- The dose-response relation was shifted by 1 $\log_{10}$ from that based on published clinical trials, in order to achieve a relationship that was more reflective of the CDC disease estimates.

The FDA is also seeking comments on the appropriateness of the risk assessment model and the parameters used to develop the model.

- Preliminary modeling of the Harvest Module did not show a great effect of salinity on the levels of pathogenic *V. parahaemolyticus*, within the range that oysters are normally harvested. Therefore this parameter was not included in our simulations.

- The study by DePaola et al., 1990. (Reference number 38) was deemed to be most appropriate for the purpose of this risk assessment, because this study was the most inclusive of seasonal changes in *V. parahaemolyticus* density in oysters from major oyster producing areas representative of the Pacific, Gulf and Atlantic Coasts. Are there other studies that should have been considered?

EXECUTIVE SUMMARY

The Food and Drug Administration (FDA) conducted a risk assessment to characterize the public health impact associated with consumption of raw oysters containing pathogenic *Vibrio parahaemolyticus.* This effort was initiated in January 1999, in response to four outbreaks occurring in the United States in 1997 and 1998 involving over 700 cases of illness, the majority of which were associated with the consumption of raw oysters. These events renewed concern for this pathogen as a serious foodborne threat to public health, given that the last outbreak in the United States occurred in 1981. In two of the 1998 outbreaks a serotype previously reported only in Asia, O3:K6, emerged as a principal cause of illness in the United States for the first time. The outbreaks also introduced uncertainty about the effectiveness of current criteria for closing and reopening shellfish waters to harvesting, and about previous FDA guidance indicating that no more than 10,000 *V. parahaemolyticus* per gram should be present in shellfish.

Input for this risk assessment was obtained from many sources, including both published and unpublished scientific literature and reports, State shellfish control authorities, the Centers for Disease Control and Prevention (CDC), the shellfish industry, the Interstate Shellfish Sanitation Conference (ISSC), and records from State Health Departments. During the development of this risk assessment model, information that was lacking was identified, and these gaps necessarily required certain assumptions to be made. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) reviewed each significant assumption included in the risk assessment.

The objectives of the risk assessment were twofold. One was to produce a mathematical model of the risk of illness incurred by consumers of raw oysters containing pathogenic *V. parahaemolyticus.* The second objective of this quantitative risk assessment was to provide FDA with information that will assist the agency with the review of current programs relating to the regulation of *Vibrio parahaemolyticus* in raw molluscan shellfish to ensure that such programs protect the public health. To accomplish this, the project sought to achieve the following: (a) evaluate the current criteria used for closing and reopening shellfish waters to harvesting, (b) evaluate preventive and intervention measures for controlling *V. parahaemolyticus* in oysters after harvest, and (c) evaluate the current guidance of 10,000 viable *V. parahaemolyticus* per gram of shellfish.

In order to develop the model, the risk assessment was divided into three modules, Harvest, Post Harvest, and Public Health. The Harvest and Post Harvest Modules differentiated two distinct time frames that affect *V. parahaemolyticus* levels in oysters harvested for raw consumption. Significant differences in oyster harvesting methods, handling practices, and climates in the United States were sufficient to distinguish five separate geographic regions (Northeast Atlantic, Mid-Atlantic, Pacific Northwest, Louisiana Gulf Coast, and the remaining Gulf Coast) and four seasons, and these were treated separately in modeling each of the modules.

The Harvest Module incorporated factors influencing the prevalence of *V. parahaemolyticus* in oysters up to the time of harvest, and identified the parameters that contribute to the likelihood

that shellfish in a growing area will contain pathogenic strains of *V. parahaemolyticus.* Quantitative modeling in this module used water temperatures as factors influencing and, therefore, potentially predicting the prevalence of pathogenic *V. parahaemolyticus* in the harvest waters and oysters.

The Post Harvest Module addressed factors associated with handling and processing of oysters after harvest, in particular those that could influence the levels of *V. parahaemolyticus* in oysters at consumption. Such factors included in the modeling of this module were ambient air temperatures at time of harvest, time from harvest that oysters remained unrefrigerated, time required to cool oysters once placed under refrigeration, and the length of time oysters are stored in refrigeration until consumption. This module also simulated intervention measures that may affect *V. parahaemolyticus* densities, such as cooling immediately after harvest, freezing, and use of mild heat treatment (5 min at 50 °C) .

The Public Health Module estimated the number of illnesses based on the levels of pathogenic *V. parahaemolyticus* at consumption, which was determined from the Harvest and Post Harvest modules. This module was further subdivided into three segments, epidemiology, consumption, and dose-response. The epidemiology segment included the number of illnesses, the severity and type of illness, the population affected, and the seasonal incidence. The consumption segment considered the number of oyster meals/servings eaten, the quantity of oysters consumed per serving, and the levels of total and pathogenic *V. parahaemolyticus* in the shellfish at consumption. The dose-response segment related the actual levels of *V. parahaemolyticus* consumed with frequency and severity of illness.

Outputs from the risk assessment model demonstrated that the single most important factor related to the risk of illness caused by this organism is the level of *V. parahaemolyticus* in oysters at the time of harvest. However, the model is based on a direct correlation between total and pathogenic *V. parahaemolyticus* levels at time of harvest. We have also assumed that pathogenic strains of *V. parahaemolyticus* grow at the same rate as non-pathogenic strains. Consequently, as the level of total *V. parahaemolyticus* increases so does the number of pathogenic *V. parahaemolyticus.* Accordingly, intervention measures aimed at controlling or reducing the levels of *V. parahaemolyticus* in oysters should have a direct bearing on controlling or reducing the risk associated with this pathogen. Water and air temperatures at time of harvest were found to be the major factors influencing the initial levels of this pathogen in oysters. Air temperature was also found to influence the growth of *V. parahaemolyticus* in oysters after harvest and, thus, the levels in oysters at the time of consumption. In oysters left unrefrigerated after harvest, *V. parahaemolyticus* rapidly multiply. The model demonstrated that these factors could have a significant impact on the likelihood of illnesses occurring. Model simulations of intervention measures indicated a significant reduction in the probability of illness when the oysters are cooled immediately after harvest and kept refrigerated. During refrigerated storage *V. parahaemolyticus* densities slowly but steadily decrease. Mild heat treatment (5 min at 50 °C) of oysters, which causes at least a 4.5 log decrease in the number of viable *V. parahaemolyticus* in oysters, practically eliminates the likelihood of illness occurring. Quick-freezing and frozen storage of oysters, which causes a 1 to 2 log decrease in viable *V. parahaemolyticus* oyster levels, also substantially reduces the probability of illness.

Earlier human feeding trials conducted in Japan showed that illnesses occurred at levels of *V. parahaemolyticus*, which were comparable to those of other bacterial strains when administered with antacids, and that the number of illnesses increased with increasing levels of pathogenic *V. parahaemolyticus*. Three different dose-response distribution models were considered for the purpose of extrapolating from these data the risk of illness associated with lower levels of exposure associated with consumption of raw oysters. Distributions of ingested dose were developed by considering the probabilistic variation of number and meat weight of oysters in a serving or eating occasion in addition to the expected variation of the density of pathogenic *V. parahaemolyticus* determined in the Harvest and Post Harvest Modules.

On the basis of all available epidemiological data, and in the absence of quantitative data to the contrary, equal virulence among pathogenic strains of *V. parahaemolyticus* was assumed for modeling purposes. Pathogenic strains of *V. parahaemolyticus* were defined as those stains possessing the ability to produce a thermostable direct hemolysin (TDH). The assessment of dose-response was developed based on this definition. This may be modified as new data become available that identify new virulence determinants.

Based on all available epidemiological information, it was assumed that all consumers are equally susceptible to infection by *V. parahaemolyticus.* The probability of illness was determined as an increasing function of ingested dose of pathogenic *V. parahaemolyticus.* Epidemiological case series data clearly suggest a greater probability of an infection leading to septicemia and death among immune compromised individuals. This is reflected in model-based estimates of severe outcome (i.e. septicemia).

The outputs from this project provide estimates of risk for illness among consumers of raw oysters (average nationwide yearly incidence of 4,750 cases per year, with a range from 1,000 to 16,000 cases - for the Gulf Coast, 25 (winter), 1,200 (spring), 3,000 (summer), and 400 (fall); for the Pacific Northwest, 15 (spring) and 50 (summer); for the Mid-Atlantic, 10 (spring) and 12 (summer); and for the Northeast Atlantic, 12 (spring), 30 (summer) and 7 (fall)). Risks increase with increasing levels of total *V. parahaemolyticus* and therefore pathogenic strains of *V. parahaemolyticus*.

Simulations on the rate of illness caused by oyster-servings where the levels of *V. parahaemolyticus* at harvest are at or above 10,000 cells/g, suggest that approximately 15% of the illnesses are associated with the consumption of oysters containing greater than 10,000 *V. parahaemolyticus* /g at time of harvest. Comparing the number of servings that cause illness to those that do not, the simulations demonstrate that on average 0.6% of the servings result in illness when *V. parahaemolyticus* levels are at 10,000 cells/g or above.

Data gaps relevant to the risk assessment have been identified, and further research is needed to narrow risk estimates and reduce the uncertainties associated with these. For example, more definitive information on other potential virulence factors, such as the capacity for invasion of the enterocytes, production of an enterotoxin, and urease production would enable better differentiation of pathogenic and non-pathogenic strains and, thereby, enable more conclusive estimates on the prevalence of pathogenic strains in shellfish waters and oysters. Another special interest for this risk assessment requiring further data is the prevalence and abundance of

pathogenic *V. parahaemolyticus* serotype O3:K6 in oysters at harvest, and also at consumption. In addition, though some studies have suggested that the immune and physiological status of an oyster could be an important factor in the prevalence of total *V. parahaemolyticus* and, therefore, in the prevalence of pathogenic *V. parahaemolyticus*, further data on this also is needed. Research needs identified by this risk assessment are summarized for the following areas:

- Environmental factors that influence distribution and abundance of pathogenic *V. parahaemolyticus* in the environment for every region and season (i.e. temperature shifts, salinity, animal passage, predation, and introduction of strains from distant areas).
- Rates of hydrographic flushing (water turnover) in shellfish harvest areas based on levels of freshwater flows, tidal changes, winds, and depth of harvesting area.
- Distribution and abundance of pathogenic *V. parahaemolyticus* in oysters at harvest.
- Growth and survival of pathogenic *V. parahaemolyticus* in oysters at various temperatures.
- Industry post harvest handling practices (i.e. time to refrigeration, cooldown periods, and length of refrigerated storage).
- Consumption patterns (frequency of raw oyster consumption from different harvest regions or seasons, and consumption by at risk groups).
- Dose-response data: how many *V. parahaemolyticus* organisms are required to cause illness, and severity of the illness.
- Potential virulence factors other than TDH (i.e. TRH, urease, enterotoxins, acid adaptation, and invasion of intestinal cells).
- Development of assays to compare virulence potential among different strains
- Role of the oyster (physiology, immune status) in levels of *V. parahaemolyticus.*
- Consumer handling of oysters prior to consumption
- Improved global public health surveillance of *V. parahaemolyticus* to identify new epidemic strains as they emerge.

This risk assessment significantly advances our ability to describe our current state of knowledge about this important foodborne pathogen, while simultaneously providing a framework for integrating and evaluating the impact of new scientific knowledge on enhancing public health.

The results of this draft risk assessment on *V. parahaemolyticus* are influenced by the assumptions and data sets that were used to develop the exposure assessment and hazard characterization. These results, particularly the predicted estimates of risk for illness among consumers of raw oysters, and the most significant parameters, which influence the incidence of illness, could change as a result of future data obtained from the Interim Control Plan and the FDA actively seeking new information, scientific opinions, or data during the public comment period. It is anticipated that periodic updates to the risk model will continue to reduce the degree of uncertainty associated with risk estimates, and that this will assist in making the best possible decisions, policies, and measures for reducing the risk posed by *V. parahaemolyticus* in raw molluscan shellfish.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF TERMS

Definitions

Case Series - study of sporadic cases of similar illness occurring over a period of time.

Depuration - the process of reducing pathogenic organisms that may be present in shellfish using a controlled aquatic environment, such as land-based tanks, as the treatment process.

Dose - the number of pathogenic *V. parahaemolyticus* consumed in oysters at one sitting.

Dose-response - the relationship of the levels of *V. parahaemolyticus* ingested with the frequency and magnitude of illness.

Gastroenteritis – inflammation of the gastrointestinal tract; symptoms include diarrhea, vomiting, or abdominal cramps, caused by an infecting organism which is present in feces.

Raw molluscan shellfish - raw (uncooked) oysters, clams, and mussels.

Outbreak - the occurrence of similar illness involving 2 or more persons not from the same household resulting from the ingestion of a common food.

Relaying - the process of reducing pathogenic organisms or deleterious substances that may be present in shellfish using the ambient environment as the treatment process, by transferring shellfish from a growing area classified as restricted or conditionally restricted to a growing area classified as approved or conditionally approved.

Sensitive subpopulation - group of people with greater vulnerability to more severe *Vibrio parahaemolyticus* disease (i.e., septicemia) as a result of some underlying state of compromised health, such as liver disease, blood disorder, or immunodeficiency.

Septicemia - a systemic disease associated with the presence and persistence of pathogenic microorganisms or their toxins in the blood.

Thermostable direct hemolysin - a toxin produced by *Vibrio parahaemolyticus* that lyses red blood cells in Wagatsuma agar .

Thermostable-related hemolysin - a toxin very similar in action and characteristics to, but genetically distinct from the thermostable direct hemolysin

ACRONYMS AND ABBREVIATIONS

CFSAN - Center for Food Safety and Applied Nutrition
GCSL - FDA Gulf Coast Seafood Laboratory, Dauphin Island
ICP - ISSC/FDA Interim Control Plan for monitoring levels of pathogenic *V. parahaemolyticus* in oysters at time of harvest
ISSC - Interstate Shellfish Sanitation Conference
MSI - Molluscan Shellfish Industry
NACMCF - National Advisory Committee for the Microbiological Criteria for Foods
NERR - National Estuarine Reserve Sites program
NBDC - National Buoy Data Center
NMFS- National Marine Fisheries Services
NOAA - National Oceanic and Atmospheric Administration
NOS - National Ocean Services
NSSP - National Shellfish Sanitation Program for control of *Vibrio vulnificus*
PCSGA - Pacific Coast Shellfish Growers Association
RAC - Risk Assessment Consortium
STORET - Storage and Retrieval of U.S. Waterways Parametric Data database

g - grams
HGMF procedure - Hydrophobic Grid Membrane Filtration procedure
h - hours
KP+ - Kanagawa-positive
min - minute
ml – milliliters
MLE - Maximum likelihood estimates
MPN - most probable number
/g - per gram
ppt - parts per thousand
TDH - thermostable direct hemolysin
TRH - thermostable-related hemolysin
Vp - *V. parahaemolyticus*
Vp_{path} - pathogenic strains of *V. parahaemolyticus*

I. INTRODUCTION

In response to four separate outbreaks that occurred in 1997 and 1998 in the United States *(21, 22)*, FDA conducted a risk assessment (RA) on the public health impact of *Vibrio parahaemolyticus* transmitted by raw oysters. Initiated in January 1999, this risk assessment focused specifically on oysters, since this was the food predominantly linked to the outbreaks.

In May 1999, FDA announced its intent to conduct a risk assessment of the public health impact of *V. parahaemolyticus* in raw molluscan shellfish in the *Federal Register (43)*. At that time, the public was invited to comment on the planned assessment and submit scientific data and information for use in the assessment. The advice and recommendations of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) were sought in May and September of 1999, on the assumptions and the risk assessment model structure to be used. During the conduct of this risk assessment, FDA solicited the technical advice and opinions of several scientific and state shellfish experts. In addition, critical review of this risk assessment model was solicited and received in December 1999 and May 2000, from members of the Interagency Risk Assessment Consortium, other government employees, and special government experts (SGE).

A chronology of the technical and scientific review involved in the development of this risk assessment is provided in Appendix I.

Over 700 cases of illness caused by *V. parahaemolyticus (21, 22)* resulted from the 1997 and 1998 outbreaks that occurred in three regions of the country, one in the Gulf Coast, two in the Pacific Northwest, and one in the Northeast. One particular serotype (O3:K6) of *V. parahaemolyticus*, previously associated only with illnesses in Asia *(109)*, was predominantly isolated from patients as the principal cause of the outbreaks occurring in the Northeast and Gulf Coast regions. It has been suggested that the 1998 Gulf Coast outbreak may have been a result of elevated water temperatures *(34)*. The influences exerted by these and other factors remain uncertain. Whatever factors were involved in these four incidents, the recurrence of *V. parahaemolyticus* outbreaks in North America and the large number of individuals affected have renewed concerns for this pathogen in raw oysters as a microbial food safety problem in the United States.

First isolated and implicated in an outbreak of food poisoning in Japan in 1950 *(48)*, *V. parahaemolyticus* has been associated with outbreaks and individual cases of illness in the United States since 1969 *(11, 32, 91, 107)*. However, since 1981 and until 1997 the only *V. parahaemolyticus* illnesses transmitted by raw molluscan shellfish in the United States have been reports of intermittent, sporadic cases, and relatively few of these occurred. The recurrence of outbreaks caused by this organism was unexpected, and the wide-ranging geography and total number of illnesses recorded were surprising. Consequently, a systematic evaluation of factors affecting *V. parahaemolyticus* in oysters and the sequence of events

leading to consumer illnesses was undertaken in order to gain a fuller understanding of the risks posed by this bacterial pathogen.

The levels of *V. parahaemolyticus* in oysters at the time of consumption can be influenced substantially by the methods chosen for harvesting and handling oysters after harvest, and these practices may vary considerably in different geographic areas and at different times of year. For example, the period of unrefrigerated storage may vary from just a few hours to more than a day, and perhaps longer in some cases. In general, the longer oysters remain unrefrigerated, the higher the level of *V. parahaemolyticus* in those oysters will become, and the higher the environmental temperature, the faster *V. parahaemolyticus* will grow. Thus, the levels of *V. parahaemolyticus* in oysters kept alive until consumption can range widely. This seemed likely to be an important element in estimating the level of risk to consumers.

The infectious dose of *V. parahaemolyticus* is not known. Based on data from volunteer studies conducted more than 25 years ago, along with data from earlier United Staets outbreaks caused predominantly by cross-contamination of cooked crabs, FDA had previously indicated that *V. parahaemolyticus* in shellfish should not exceed a level of 10,000 viable cells per gram *(64)***.** However, data obtained during the recent outbreaks indicated that fewer than 10,000 *V. parahaemolyticus* per gram were present in oysters obtained from implicated harvest areas during the same period *(21).* In fact, the overall levels of *V. parahaemolyticus* found in some oysters from implicated harvest sites were as low as 100 and often less than 1,000 cells per gram. In view of this, and the fact that not all *V. parahaemolyticus* strains are pathogenic, it seemed possible that the earlier guidance alone may not be sufficient to protect consumers from *V. parahaemolyticus* illness associated with raw oysters.

The risk assessment has focused on raw oysters because these were the predominant seafood implicated in the 1997 and 1998 outbreaks *(5, 21, 22).* This endeavor investigated the prevalence of *V. parahaemolyticus*, the extent of consumer exposure to the organism, the resulting public health impact, and the effects of several post harvest treatments to control exposure to this hazard in raw oysters. A systematic evaluation of the available scientific information was conducted to assist public health officials in assessing different potential control measures and developing food safety guidance and policies. The risk assessment had two main objectives: (1) create a mathematical model and assess the current risk of becoming ill due to the consumption of pathogenic *V. parahaemolyticus* in raw oysters; and (2) develop a comprehensive and current scientific framework, which will assist the agency with the review of current programs relating to the regulation of *V. parahaemolyticus* in raw molluscan shellfish to ensure that such programs protect the public health.

Exposure is a function of the prevalence of *V. parahaemolyticus* in the oysters consumed and the consumption patterns of the population. Thus, the number of pathogenic *V. parahaemolyticus* in oysters at consumption, and what leads to this number, is critical exposure information. Accordingly, the scope of the risk assessment specifically attempted to address the following questions:

- What is the frequency of occurrence of pathogenic strains of *V. parahaemolyticus* in the shellfish waters, and what parameters (e.g., water temperature, salinity, turbidity, and

nutrient profiles) can be used as indicators of the presence of the organism in growing waters?

- What is the frequency of occurrence of pathogenic strains of *V. parahaemolyticus* in oysters, and what are the numbers of viable pathogenic organisms at time of consumption? How are levels present in the bivalves at the time of consumption related to the initial levels in the growing waters?
- What is known about the dose-response relationship from outbreak, epidemiological, animal and other studies? What are the differences in dose-response relations among different strains and serotypes of *V. parahaemolyticus*, and among consumers with different susceptibilities?
- What is the role of post harvest handling that may be influencing the numbers of *V. parahaemolyticus* in oysters? What reductions in risks can be achieved by intervention strategies such as depuration, (the process by which shellfish are cleansed in seawater in tanks), or relaying (the process by which shellfish are cleansed by transferring them to clean shellfish growing areas).
- What is the adequacy of current scientific knowledge and where should future research be focused to reduce the uncertainty in the risk estimate?

Thus, the risk assessment encompassed the relationships between oysters, *V. parahaemolyticus*, and illnesses, and exposure to pathogenic *V. parahaemolyticus*. The risk assessment attempted to evaluate the following factors:

- Evidence for increased risks from certain newly emerging strains causing outbreaks, such as serotype O3:K6.
- The effectiveness of potential strategies for limiting exposure of the public to raw oysters containing pathogenic *V. parahaemolyticus* and reductions in risks, which might be achieved by intervention strategies such as rapid cooling, quick freezing, mild heat treatment, depuration and relaying.
- Current criteria for opening and closing shellfish harvest waters.
- Current FDA guidance that shellfish should contain less than 10,000 *V. parahaemolyticus* per gram.

II. RISK ASSESSMENT PROCESS

Risk assessment provides the scientific basis for risk analysis. It is the process of determining the likelihood that exposure to a hazard, such as a foodborne pathogen, will result in harm or disease; it helps characterize the nature and magnitude of risks. Risk assessments also assist regulators in decision making on food safety guidance and policies by providing a systematic evaluation of the state of knowledge related to the hazard including the degree of uncertainty. The *V. parahaemolyticus* risk assessment (VPRA) process illustrated in this document adhered to the framework proposed by the CODEX Committee on Food Hygiene *(68)* and by the National Advisory Committee on Microbiological Criteria for Foods *(103)*, which involves four steps: Hazard Identification, Exposure Assessment, Hazard Characterization/Dose-Response, and Risk Characterization.

This risk assessment utilized quantitative risk assessment modeling, which is a mathematical process used to evaluate the likelihood of adverse human health effects occurring following exposure to a pathogenic microorganism. Or more simply stated, it describes what we know and how certain we are of what we know. The risk is expressed as a mathematical statement of the chance of illness or death after exposure to a specific pathogen and it represents the cumulative probabilities of certain events happening and the uncertainty associated with those events. Quantitative risk assessment modeling is a relatively new approach to the field of microbial risk. The data are represented as large sets of numbers called distributions rather than as point estimates, which offer several potential advantages over traditional approaches. One advantage is that distributions may represent the spread of real world data more accurately than point estimates like a mean. Distributions can also reflect the presence of uncertainty in the data. Another potential advantage is that modeling allows risk assessors to test which factors are most important in determining the magnitude of a risk or what effect control measures will have on a risk. Quantitative models are also flexible; inputs and model components can be changed readily as new data become available.

Determinants of the hazard of *V. parahaemolyticus* illness due to consumption of oysters containing this pathogen, include: the initial levels of pathogenic *V. parahaemolyticus* in the oysters at harvest, the effect of normal handling and processing on these levels, and the pathogen's capability to multiply and reach an infectious level in the food prior to consumption. Taking into account these determinants, the risk assessment was divided into three different modules within the CODEX framework: Harvest, Post Harvest, and Public Health Modules. The Public Health Module was further subdivided into three segments: epidemiology, consumption, and dose-response, even though dose-response forms the Hazard Characterization section of the risk assessment.

III. HAZARD IDENTIFICATION

Hazard identification is the identification of biological, chemical, or physical agents capable of causing adverse health effects that may be present in a particular food or group of foods. *V. parahaemolyticus* is a Gram-negative, halophilic bacterium that occurs naturally in estuaries and is recognized as an important bacterial seafood-borne pathogen throughout the world. The organism can cause an acute gastroenteritis and, on rare occasions, septicemia. The minimum infectious dose is not known. It is normally present in many seafoods, including fish, crustaceans, and molluscan shellfish. However, not all strains of *V. parahaemolyticus* cause illness and, in fact, pathogenic strains very rarely have been isolated from the environment or seafood. Apparently non-pathogenic *V. parahaemolyticus* are far more prevalent in nature. Several different virulence traits have been associated with the pathogenesis of *V. parahaemolyticus* strains. These include their ability to: a) produce a thermostable direct hemolysin (TDH) *(96)*, b) to invade the enterocytes *(3)* and c) to produce an enterotoxin *(61)*. However, these last two characteristics are not normally investigated in the environmental or clinical isolates, and the only trait known to reliably distinguish pathogenic from non-pathogenic *V. parahaemolyticus* is the production of a thermostable direct hemolysin (TDH). Pathogenic strains possess a *tdh* gene and produce TDH, and non-pathogenic strains lack the gene and the trait *(96)*. Non-pathogenic strains, as identified by the absence of the TDH, are predominantly found in the environment.

The most common clinical manifestation of *V. parahaemolyticus* is gastroenteritis, which is usually a self-limited illness with moderate severity and short duration *(11, 12, 57)*. However, on rare occasions, infection can result in septicemia that can be life threatening *(57, 83)*. Gastroenteritis, due to a specific organism, is an inflammation of the gastrointestinal tract, characterized by diarrhea, vomiting, or abdominal cramps, and that organism is isolated from a stool sample. Septicemia is a systemic disease, characterized by fever or hypotension, and the organism is isolated from the blood. Patients with septicemia often have underlying medical conditions *(83)*.

IV. EXPOSURE ASSESSMENT

Exposure assessment is the determination of the likelihood of ingesting pathogenic *V. parahaemolyticus* by eating raw molluscan shellfish harboring the organism and the amount of pathogenic *V. parahaemolyticus* present when consumed. Exposure assessment is subdivided into Harvest, Post Harvest, and the epidemiology and consumption segments of the Public Health Module.

Harvest Module

The Harvest Module identifies the parameters contributing to the likelihood that shellfish in a growing area will contain disease-causing (pathogenic) strains of *V. parahaemolyticus* and the levels in which they're found. These parameters are listed below.

Routes of introduction of *V. parahaemolyticus* into shellfish growing areas and in shellfish

Vibrio spp. are found in the estuarine environment in the tropical to temperate zones. Several studies have been published on the concentration of *V. parahaemolyticus* in shellfish growing areas *(35, 38, 70, 73, 74, 76, 77, 89).* There are several pathways by which *V. parahaemolyticus* strains may be introduced into shellfish growing areas. *V. parahaemolyticus* may originate or new strains may be introduced naturally by terrestrial and aquatic animals, or through human activities such as "relaying" shellfish or releasing ballast water. Terrestrial and aquatic animals (including plankton, birds, fish, reptiles) may harbor virulent strains of *V. parahaemolyticus* and may play a role as intermediate hosts and vehicles for spread *(118). V. parahaemolyticus* has been isolated from a number of fish species where it is associated primarily with the intestinal contents *(101). V. parahaemolyticus* can be introduced into non-contaminated areas by relaying shellfish prior to commercial harvesting.

Ship ballast release may be a potential mechanism of introducing *V. parahaemolyticus* into a particular environment. Most cargo ships must carry substantial quantities (millions of gallons) of ballast water to operate safely when they are not carrying cargo. Cargo ships take on ballast water from the body of water in which the ship originates. Having taken water on board, it is normally retained until the ship is about to load cargo, at which point ballast water is discharged. During de-ballasting, organisms picked up from one port could be introduced into the loading port. Ship ballast may have spread the epidemic strain of *V. cholerae* to the U.S. Gulf of Mexico *(93).* Strains of *V. cholerae* indistinguishable from the Latin American epidemic strain were found in non-potable water taken from a cargo ship docked in the Gulf of Mexico. The same could occur for *V. parahaemolyticus*.

Sewage discharge may indirectly influence the densities of *V. parahaemolyticus* present in shellfish growing areas *(141).* For example, densities of *V. parahaemolyticus* in the water column in Narragansett Bay, Rhode Island were correlated with fecal coliforms from sewage; however, the effect of sewage was an indirect one mediated by stimulation of zooplankton with

which the *V. parahaemolyticus* were associated. Laboratory studies showed that nutrients in the sewage did not directly increase *V. parahaemolyticus* levels *(141)*. Other reports have shown that organic matter does have an effect on growth and survival of the organism *(121)*. In another study, the distribution *of V. parahaemolyticus* in sediments in Boston Harbor was independent of densities of fecal coliforms *(120)*.

Prevalence and persistence of *V. parahaemolyticus* in shellfish and in shellfish growing areas

Once introduced, a number of factors are relevant to whether *V. parahaemolyticus* will become established. These include interactions of environmental conditions, species and physiology of the shellfish, and the genetics of the microorganism. Certain areas may have more favorable environmental conditions that support establishment, survival, and growth of the organism. Predictive factors to be considered in determining the prevalence of *V. parahaemolyticus* include temperature (including El Niño and La Niña weather patterns), salinity, zooplankton, tidal flushing (including low tide exposure of shellfish) and dissolved oxygen *(4, 49, 70, 137)*.

Warmer temperatures and moderate salinities, especially those prevailing during the summer months, favor the growth and survival of *V. parahaemolyticus (31, 65, 101, 147)*. Most of the shellfish-borne illnesses caused by this organism also occur in the warmer months. The Centers for Disease Control and Prevention (CDC) randomly selected seven of the 76 (nine percent) existing Texas Department of Health monitoring sites for environmental conditions in Galveston Bay, and compared water temperature and salinity levels before and during the 1998 outbreak, with environmental data recorded over the previous five years *(34)*. They demonstrated a significant difference in mean values. During May 1998, water temperatures were 81^{o} Fahrenheit (F) compared with 76^{o} F for the previous five years. In June, water temperatures were 85^{o} F, compared with 83^{o} F for the previous five years. Significantly less rainfall than usual, during April (0.59 inches) and May (0.02 inches) preceding the outbreak, causing extreme drought conditions in Texas, resulted in markedly increased salinity levels in Galveston Bay. During May, salinity levels were 18.3 parts per thousand (ppt) compared with 8.4 ppt for the previous five years. During June, salinity levels were 21 ppt compared with 9.1 ppt for the previous five years. It is therefore possible that environmental factors such as increased temperature and salinity levels, known to promote growth of *V. parahaemolyticus*, may have contributed to this outbreak. Elevated temperatures were also suspected to have played a role in the 1997 outbreak on the West Coast *(22)*.

Another variable that must be considered is that *V. parahaemolyticus* often "over-winters" (survives the winter) in the sediment and is absent from the water column and oysters during the winter months *(69, 75, 136)*. During the summer, shellfish often have levels *of V. parahaemolyticus* from 10- to 100-fold greater than those in the water *(38, 73)*; therefore, sediment should be the preferred samples for monitoring during the winter and shellfish should be the preferred samples for monitoring during the summer. Under extreme environmental conditions, *Vibrio* species, including *V. parahaemolyticus*, may enter a "viable but non-culturable (VBNC) phase" in marine waters and could be missed by traditional cultural methods *(15, 24, 110, 146)*. This issue remains a controversial one. Methods such as gene probes

developed by the FDA are capable of detecting most virulent strains and are useful in monitoring programs *(51).*

V. parahaemolyticus favors the presence of particulates, zooplankton and other chitin sources *(70, 102, 137).* Microorganisms are incorporated into shellfish by filter feeding. Factors that favor active filter feeding by shellfish increase the probability that shellfish in a given area will take up the pathogen *(100).* Shellfish species and physiology (e.g., sexual maturity, immune function, metabolic state) can affect survival and growth of disease-causing *Vibrio* spp. within shellfish. There is evidence that the immune status of the shellfish may play an important role in the prevalence and persistence of the microorganism *(45, 85, 86, 111, 138).* There also appear to be seasonal differences in the oyster's cellular defense system. A recent study showed that the bactericidal activity of hemocytes (oyster blood cells) was greater in summer than in winter *(50).* Certain factors, such as the oyster parasite *Perkinsus marinus*, play a role in the affinity of bacteria for shellfish tissue and the ability of oyster hemocytes to kill the internalized organisms *(85, 86, 126).* Factors, such as spawning or adverse environmental conditions (e.g., the presence of chemicals in the environment: tributyltin oxide, polycyclic aromatic hydrocarbons, wood preservative leachates), that reduce or stop filter feeding in shellfish, or cause selective feeding (e.g., new nutrient sources) may prevent or delay incorporation of *V. parahaemolyticus* into shellfish by affecting oyster physiology and possibly affect oyster-bacterial interactions *(124, 142, 143).*

Persistence of virulent strains of *V. parahaemolyticus* in shellfish in the environment may be dependent on several parameters. Whether virulent and non-virulent strains are affected in a similar fashion by environmental and other factors is unknown. The presence of the urease gene may provide a competitive environmental advantage over other strains allowing access to a wider range of nutrients *(1).* Urease-positive strains have been identified as a predominant cause of *Vibri*o-associated gastroenteritis on the U.S. West Coast and Mexico *(1).* The presence of a pathogenicity island (a physical grouping of virulence-related genes) in *V. parahaemolyticus* may foster rapid microevolution, promote growth and survival, and result in transmission of factors, such as those responsible for virulence, to other strains (horizontal gene transfer) *(47, 62, 63).* In addition, bacteriophages may genetically alter vibrios *(14, 62).*

The distribution and variation in levels of virulent *V. parahaemolyticus* in shellfish and among shellfish growing areas may need to be determined before harvest because many of the described factors may have contributed to higher concentrations in certain areas. During the 1998 outbreaks, the Pacific Northwest shellfish harvested from the Hood Canal area of Washington were responsible for 32 of 48 (67 %) of the illnesses in the State of Washington *(132).* In the Gulf Coast, 20 of 30 harvest sites were implicated. In the Atlantic Northeast region, Oyster Bay Harbor (Area 47) was the only area implicated in the outbreak of that region *(21).*

Modeling of the Harvest Module

Although a number of factors have been identified as potentially affecting the levels of pathogenic *V. parahaemolyticus* in oysters at time of harvest, there are not sufficient quantitative data available to incorporate all of these factors into a predictive model. To incorporate an environmental factor into the simulation, as a predictor of *V. parahaemolyticus* densities at harvest, it is necessary to identify both the relationship of *V. parahaemolyticus* densities to the parameter of interest and the regional and temporal variation of the parameter within the environment. Moreover, due to the relatively low prevalence of pathogenic *V. parahaemolyticus* and limitations of current methods of detection, the distribution of pathogenic *V. parahaemolyticus* is not well understood. A critical issue in the development of the Harvest Module simulation is the use of the estimated distribution of total *V. parahaemolyticus* densities to bridge this data gap and derive an estimate of the distribution of pathogenic *V. parahaemolyticus* densities in oysters at harvest. Figure IV-1 is a schematic depiction of the parameters considered in modeling the Harvest Module. Preliminary modeling demonstrated that the parameter, water salinity is not as strong a determinant of *V. parahaemolyticus* levels as water temperature, and therefore is represented as a dotted bubble.

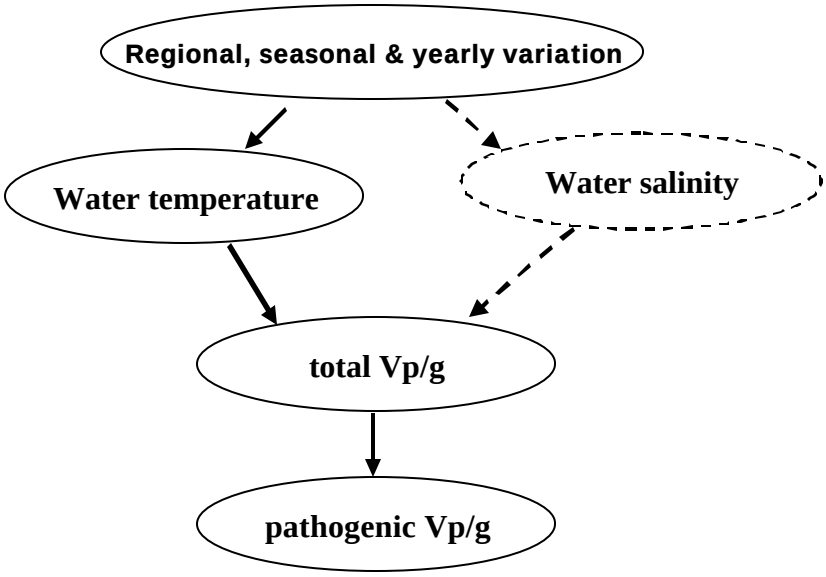

Figure IV-1. Schematic depiction of the Harvest Module of the *V. parahaemolyticus* (Vp) risk assessment model.

Effect of water temperature and salinity on total *V. parahaemolyticus* densities

The best available data on the relationship of total *V. parahaemolyticus* densities in oysters (and water) versus water temperature and salinity is found in the study by DePaola *et al*, 1990 *(38).* This study was conducted throughout an entire year with collection of samples from all four regions of the country (i.e., Northeast, Gulf Coast, Mid-Atlantic, and Pacific Northwest). A total of 65 paired samples of oyster and water were analyzed for total *V. parahaemolyticus* by a membrane filtration method. While there have been several other surveys of *V. parahaemolyticus* between 1982 to 1995, these studies are typically limited to specific regions and/or seasons , and few have reported quantitative data. These studies are summarized below.

Kelly and Stroh *(78)* examined *V. parahaemolyticus* frequency in natural and cultivated oysters from British Columbia and isolated *V. parahaemolyticus* from 44% of natural and 21% of cultivated oysters under warm conditions (July and August) but did not find *V. parahaemolyticus* in March and April.

Kelly and Stroh *(79)* also reported an association with *V. parahaemolyticus* illness and *V. parahaemolyticus* density in the estuarine waters of British Columbia. *V. parahaemolyticus* was isolated in 11-33% of water samples collected during the summer with peak densities of 70 cfu/ml. Oysters were not examined.

Kaysner *et al. (74)* sampled water, sediment and oysters of Willapa Bay, WA during August when salinity ranged from 23.6-30.5 ppt and temperature 15.5-22.6°C. Highest densities ($\log_{10}$ MPN/g) were found in sediments (1.6-5.4), followed by oysters (1.5-4.0) and water (0.5-3.0); a similar trend was observed with frequency of isolation.

Tepedino *(130)* surveyed Long Island oysters from October to June and found 33% to contain *V. parahaemolyticus* with an MPN range of 3.6-23/g.

Hariharan *et al. (55)* conducted a year long survey of Prince Edward Island, Canada mussels and oysters and *V. parahaemolyticus* was isolated from 4.7% and 6.7%, respectively.

Chan *et al. (23)* examined *V. parahaemolyticus* levels in seafood from Hong Kong from June through October. Mean *V. parahaemolyticus* densities in oysters (harvest), mussels (market) and clams (market) were 3.4×10^4, 4.6×10^4, and 6.5×10^3 per gram, respectively.

Kiiyukia *et al. (81)* enumerated *V. parahaemolyticus* in water and sediments of Japan. They isolated *V. parahaemolyticus* in 2/8 market oyster samples but did not enumerate *V. parahaemolyticus* in oysters.

Ogawa *et al. (108)* investigated the ecology of *V. parahaemolyticus* in Hiroshima Bay from July 1987 through June 1988. The highest incidence of detectable *V. parahaemolyticus* (68.8%) was found from May to October when water temperature ranged from 19.3 to 22.0°C. *V. parahaemolyticus* levels in oysters were seasonal and ranged from $10^3 - 10^1$/100g *(108).* This study also compared favorably with the DePaola *et al.* 1990 study *(38).*

DePaola *et al. (40)* had previously evaluated 4 methods for enumeration of *V. parahaemolyticus* in natural seawater and oysters and found considerable variability between methods for *V. parahaemolyticus* recoveries; highest recoveries were obtained with a method using filtration through a hydrophobic grid membrane.

DePaola *et al.* in 1990 *(38)* enumerated *V. parahaemolyticus* (hydrophobic grid method) in seawater and oysters samples collected seasonally from May 1984 through April 1985 from shellfish growing areas from the Pacific, Gulf and Atlantic Coasts. Seasonal and geographical distributions of *V. parahaemolyticus* were related to water temperature, with highest densities in samples collected in the spring and summer from the Gulf Coast.

We considered the study by DePaola *et al. (38)* to be most appropriate for the purpose of quantitative risk assessment of *V. parahaemolyticus* illness from consumption of U.S. oysters. This study, which is the most comprehensive regional/seasonal study available, examined seasonal changes in *V. parahaemolyticus* density in oysters from major oyster producing areas representative of the Pacific, Gulf and Atlantic Coasts *(38).* Studies reporting only presence or absence of detectable *V. parahaemolyticus* are of limited value for quantitative risk assessment *(55, 78, 79, 81)*. Of the additional studies available reporting quantified *V. parahaemolyticus* densities in oysters, samples were either obtained from a single estuary *(23, 55, 74, 78, 79, 81, 130)*, were not seasonal *(23, 74, 130)*, or did not report salinity and temperature *(23, 130).* Differences in methodology used by the various investigators may also have affected *V. parahaemolyticus* recoveries and complicate comparisons between studies. *V. parahaemolyticus* levels observed in oysters from Long Island, NY *(130)* were similar to those reported by DePaola *et al. (38)* from the Northern Atlantic Coast during the fall, winter and spring. Kaysner *et al. (74)* observed higher *V. parahaemolyticus* densities in Willapa Bay, WA in August than reported on the Pacific Coast during the summer by DePaola *et al. (38).* This difference may have been due to the small number of samples (N=4) collected from the Pacific Coast during the summer by DePaola *et al. (38),* or to favorable environmental conditions for *V. parahaemolyticus* abundance in Willapa Bay during the study by Kaysner *et al. (74).* Including the data from other studies of the Atlantic *(130)* and Pacific Coasts *(74)* would increase the sample size. However, these studies employed different methodology than that used by DePaola *et al. (38)* and inclusion of these data could bias comparisons between other seasons or regions that did not include data from these individual estuaries using different methods. Specifically, due to differences in method error associated with various analytical methods, statistical analysis of pooled data must account for the differences in variation of observed measurements according to the analytical methods used. Although this may be readily accomplished, the precision of estimating trends is not necessarily increased due to the necessity of estimating multiple sources of variation. Furthermore, lack of precise estimates of method error makes it difficult to estimate the population variation of *V. parahaemolyticus* densities (i.e., true variation in the absence of method error). Consequently, to maintain consistency only data from DePaola *et al. (38)* was used in the harvest module of this risk assessment.

The distributions of total *V. parahaemolyticus* densities in water and oyster samples were positively skewed. This is consistent with the almost universal observation that microbial populations in foods are lognormally distributed. Therefore the logarithm of the density being more normally distributed was regressed against temperature and salinity. *V. parahaemolyticus*

was not detected in a relatively large proportion of samples (e.g. 19 of 61 oyster samples (31%)). Some of these samples are likely to have been false-negatives due to limitations of the method. In order to avoid upward bias of predicted levels at low temperatures the estimate of the regression line of $\log_{10}$ total *V. parahaemolyticus*/g oyster meat was obtained by the censored or Tobit regression method. The Tobit regression is a maximum likelihood procedure with likelihood reflecting both the probability of obtaining a nondetectable outcome at a given temperature as well as the probability distribution of observable densities given that a sample has detectable *V. parahaemolyticus.* The effect of this likelihood structure is to weight the influence of nondetectable outcomes on estimated trends differently in comparison to samples with quantifiable densities. The influence of nondetectable outcomes is based on the probability of the density of a sample falling below a fixed limit of detection rather than the assumption that a nondetectable measurement corresponds to an observed and quantifiable density at the limit of detection or one-half the limit of detection as is commonly assumed.

In the reanalysis of the DePaola *et al.* study *(38)*, the effect of temperature on mean $\log_{10}$ total *V. parahaemolyticus* densities was found to be approximately linear over the range of environmental water temperatures. The presence of a quadratic effect in temperature was not evident (i.e., not significant). With regard to salinity, a quadratic effect was found to be significant, suggesting that *V. parahaemolyticus* increase with increasing salinity up to an optimal level and then decrease with increasing salinity thereafter. There was no significant interaction between temperature and salinity evident based on the data. Consequently, the best fitting model obtained was of the form

$$\log(Vp/g) = \alpha + \beta * TEMP + \gamma_1 * SAL + \gamma_2 * SAL^2 + \epsilon$$

where TEMP denotes temperature in °C; SAL denotes salinity in parts per thousand (ppt); α, β, γ_1, and γ_2 are regression parameters for temperature and salinity effects on mean $\log_{10}$ densities, and ϵ is a random normal deviate with zero mean and variance σ^2 corresponding to the combined effects of population and method error variation.

The resulting parameter estimates were

$$\alpha = -2.6$$
$$\beta = 0.12$$
$$\gamma_1 = 0.18$$
$$\gamma_2 = -0.004$$
$$\sigma^2 = 1.0$$

The estimated relationships between total *V. parahaemolyticus* densities in oysters versus water temperature and salinity are shown in Figures IV-2 and IV-3, respectively.

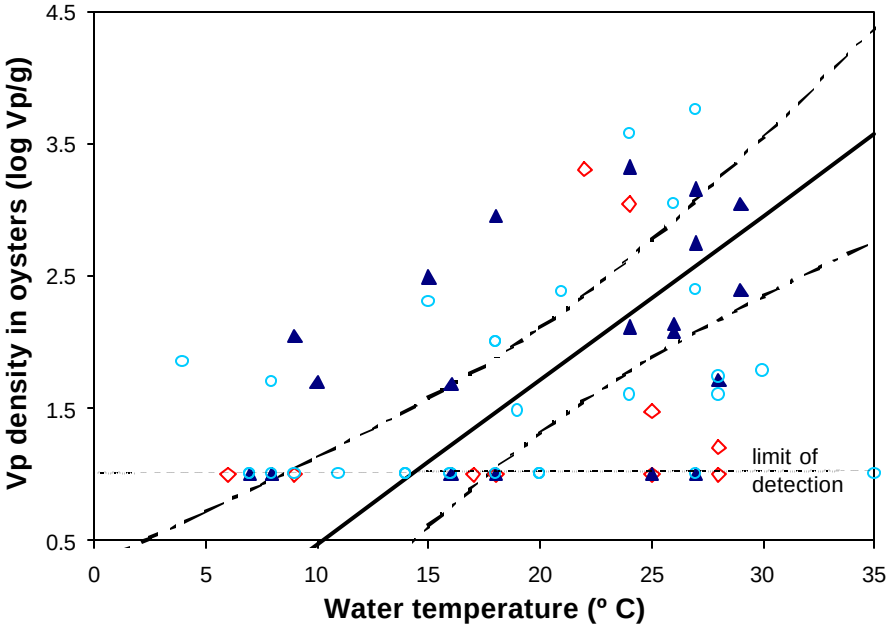

Figure IV-2. Observed log10 V. parahaemolyticus (Vp) densities in oysters versus water temperature at different salinities.
(<10 ppt (◊), 10 to 20 ppt (▲) and >20 ppt (○) in comparison to model predicted effect of temperature on mean $\log_{10}$ density (solid line) and 95% confidence limits (dashed lines) at salinity of 22 ppt).

Both salinity and temperature effects were significant based on the regression. The variation of observed values about the predicted mean regression line shown in Figure IV-2 is attributable to the effects of salinity as well as the variation about the mean due to population variation and method error. This regression line gives the predicted mean levels versus temperature at a predicted optimal salinity of 22 parts per thousand (ppt). Similarly the variation of the observed data about the regression curve (parabola) for salinity effect shown in Figure IV-3 is partially attributable to differences in water temperature in addition to population and method error variation about the mean.

Extremes of salinity below 5 ppt are known to be detrimental to survival of *V. parahaemolyticus*. However, the influence of salinity within a range of moderate environmental salinities (i.e., 5-35 ppt) is not as clear. Based on the regression analysis, a quadratic relationship for *V. parahaemolyticus* densities versus salinity within the 5-35 ppt range is consistent with the DePaola *et al.* data *(38).* However, this projected effect of salinity is not as strong as that of temperature. Within a broad range around the optimal salinity of 22 ppt, the results of the regression suggest that the differences in salinity actually encountered in oyster harvesting have relatively little effect on the *V. parahaemolyticus* population (Figure IV-4).

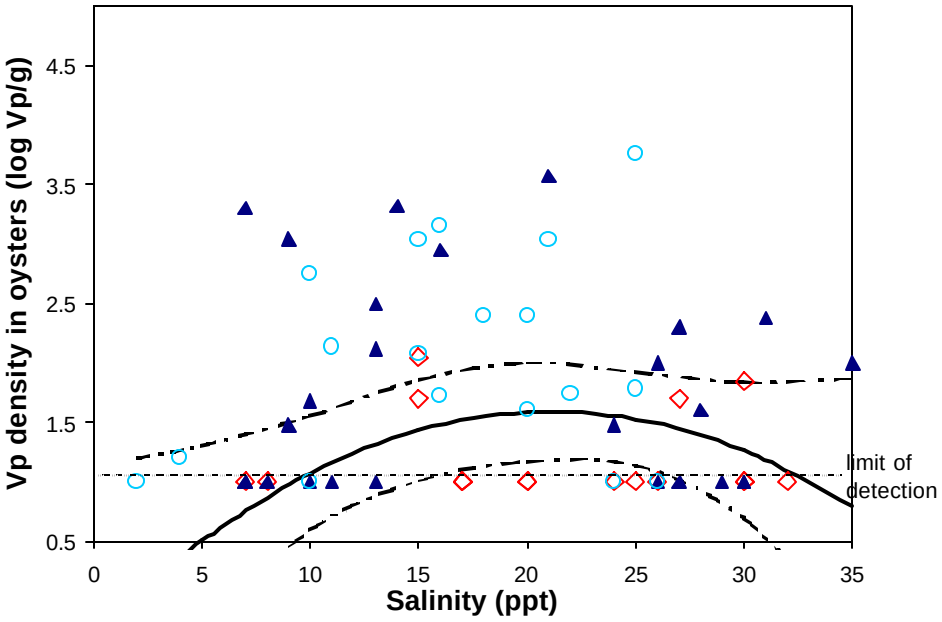

Figure IV-3. Observed log10 *V. parahaemolyticus* (Vp) densities in oysters versus salinity at different temperatures.
(<15° C (◊), 15 to 25° C (▲), and >25° C (○) in comparison to model predicted effect of salinity on mean log_{10} density (solid line) and 95% confidence limits (dashed lines) at temperature of 19° C).

Clearly, in order to predict the distribution of *V. parahaemolyticus* densities at harvest based on the regression and the projected influence of water temperature and salinity in the environment, representative distributions of water temperature and salinity need to be estimated. Based on near-shore buoy data available from the National Buoy Data Center, regional and seasonal distributions of water temperature were available. However, representative data concerning the variation of salinity in shellfish growing areas were not identified. Consequently the effect of salinity was not incorporated into the present simulation.

Two considerations suggest that neglecting the effect of salinity does not adversely affect the predictive value of a model based on temperature alone. First, as shown in Figure IV-4, predicted mean *V. parahaemolyticus* densities vary by less than 10% from the optimal (maximum) density as salinity varies from 15 to 30 parts per thousand (ppt). Secondly, measurements of oyster liquor salinity at the retail level *(44)*, which are strongly correlated with salinity of harvest waters *(44)*, suggest that oysters may be harvested from the more saline areas of the estuaries year round. The mean oyster liquor salinity in the ISSC/FDA survey was found to be 24 ppt with a standard deviation of 6.5 ppt based on 249 samples. The study was conducted year round with samples obtained from all regions of the country. These two considerations suggest that the effect of variation of salinity on predicted distributions of *V.*

parahaemolyticus densities would be minor. Variations in salinity between 15 and 30 ppt would increase the variance of the predicted distribution by only a small amount.

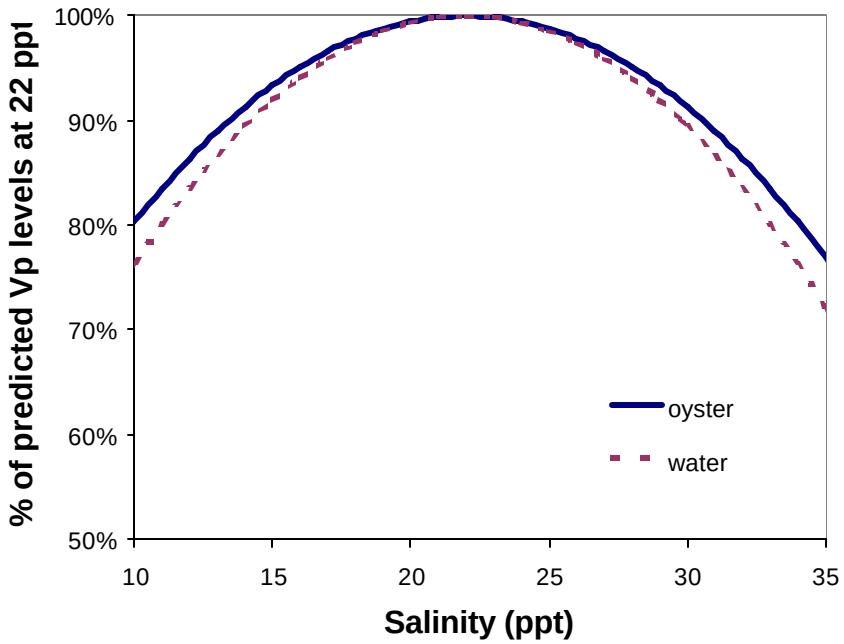

Figure IV-4. Effect of salinity on predicted mean log10 *V. parahaemolyticus* (Vp) density in oysters and water relative to predicted density at optimal salinity (22 ppt).

Neglecting the effect of variations in salinity in the simulation can be accomplished in either of two ways. Either salinity can be fixed to a mean value (i.e., 22 ppt) in the regression relationship derived above or the prediction of *V. parahaemolyticus* densities can be based on a regression analysis of the DePaola *et al.* data *(38)* with water temperature as the only effect in the model. With water temperature as the only effect the regression equation is:

$$\log(Vp/g) = \alpha + \beta * TEMP + \varepsilon$$

where TEMP denotes temperature in ° C, α and β are regression parameters for temperature effect on mean $\log_{10}$ densities, and ε is a random normal deviate with zero mean and variance σ^2 corresponding to the combined effects of population and method error variation.

Parameter estimates obtained based on the Tobit estimation method are

$$\alpha = -1.03$$
$$\beta = 0.12$$
$$s^2 = 1.1$$

Based on the data, the estimate of the variance about the mean (σ^2) is an inflated estimate of population variation due to method error. An estimate of population variation about the mean is obtained by subtracting out an estimate of the method error. The membrane filtration method used in the DePaola *et al.* study *(38)* was the HGMF procedure developed by Watkins *et al. (140)* and latter revised by Entis *(41).* When all suspect colonies are tested for confirmation, the precision of the HGMF method has been shown to be somewhat greater than the 3 tube MPN (most probable number) procedure *(41, 140).* In the DePaola *et al.* study *(38)*, enumeration of *V. parahaemolyticus* colonies was based on testing of five suspect colonies. Consequently, enumeration was not as precise as possible and overall method error associated with estimating *V. parahaemolyticus* densities may have been more comparable to that of a 3 tube MPN procedure. An estimate of the method error variance of the 3 tube MPN procedure is 0.35 *(39)* and this value was considered a reasonable estimate of the method error for the DePaola *et al.* study *(38).*

The predicted mean log *V. parahaemolyticus* level versus temperature for the temperature only regression is shown in Figure IV-5. Clearly, this relationship is comparable to that which would be obtained by fixing the salinity to a near optimal value (22 ppt) in the prediction equation based on both water temperature and salinity. The temperature only regression was used to model the relationship between temperature and density of total *V. parahaemolyticus* at time of harvest.

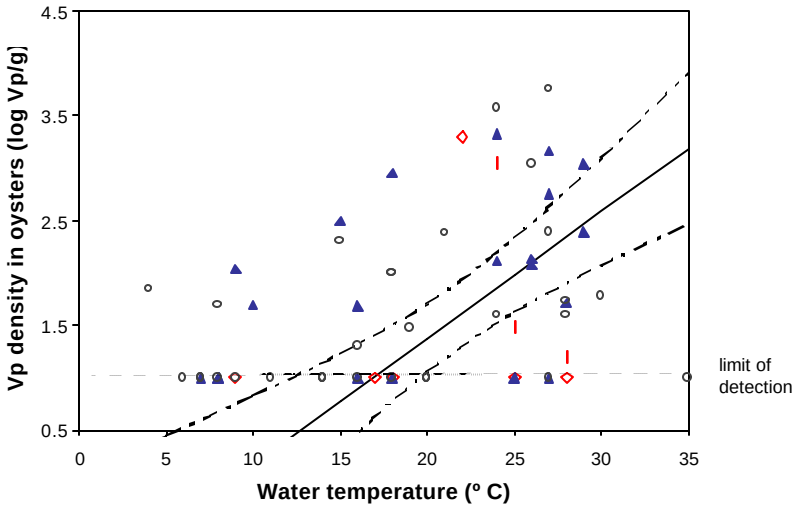

Figure IV-5. Observed log10 *V. parahaemolyticus* (Vp) densities in oysters versus water temperature at different salinities.
(<10 ppt (◊), 10 to 20 ppt (▲) and >20 ppt (○) in comparison to predicted $\log_{10}$ densities (solid line) and 95% confidence limits (dashed lines) based on temperature only regression model).

Water Temperature Distributions

Regional and seasonal distributions of water temperatures were developed based on accumulated records from coastal water buoys (National Buoy Data Center (NBDC) data). Seasons were defined by calendar month: winter (January through March), spring (April through June), summer (July through September), and fall (October through December). For each region and season a shallow water buoy was selected as being representative of the water temperature distribution for oyster harvest areas within that region/season combination. The available database for most buoys has hourly water temperatures from 1984 up to the present, with occasional data gaps due to instrumentation malfunction. The correlation between water temperature and the ambient air temperature that oysters are subject to after they are harvested was accounted for by selecting buoys for which air temperature records were also available.

Considering that oyster harvesting outside of the Pacific Coast region commences early in the morning and ends mid or late afternoon, the daily water temperature recorded at noon was considered to represent an average daily temperature.

The distribution of these "average" temperatures within a given region and season varies from year-to-year with wider variations occurring during the transitional seasons of spring and fall.

Within a given year, the distribution of the noontime water temperature was found to be unimodal within a given range. This empirical distribution is adequately approximated as a normal distribution provided that no weight is given to implausible values outside the historical range of values that may be expected. Differences in these distributions from one year to the next are evident in the buoy data. We have characterized this year-to-year variation in the water temperature distributions by calculating the central tendency and variation in both the mean and standard deviation of these distributions. The buoys selected and the summary statistics calculated are shown in Table IV-1.

Table IV-1. Summary statistics (mean, variance and correlation) of the year-to-year variation in the mean and standard deviation of noontime water temperature distributions for different regions and seasons

Region	Seasonal Water Temperature Distributions (°C)			
	Winter (Jan - March)	**Spring** (April - June)	**Summer** (July - September)	**Fall** (October - December)
Northeast Atlantic (Ambrose buoy, NY harbor)	mean(μ) [a] = 4.51 mean(σ) = 1.23 variance(μ) = 1.04 variance(σ) = 0.23 corr(μ,σ) = -0.14	mean(μ) = 12.0 mean(σ) = 4.2 variance(μ) = 0.74 variance(σ) = 0.34 corr(μ,σ) = 0.57	mean(μ) = 20.7 mean(σ) = 1.34 variance(μ) = 0.86 variance(σ) = 0.22 corr(μ,σ) = -0.25	mean(μ) = 12.0 mean(σ) = 3.37 variance(μ) = 0.73 variance(σ) = 0.36 corr(μ,σ) =-0.08
Mid-Atlantic (Thomas Point Lighthouse buoy, Chesapeake Bay)	mean(μ) = 3.92 mean(σ) = 1.92 variance(μ) = 1.0 variance(σ) = 0.21 corr(μ,σ) = -0.31	mean(μ) = 16.8 mean(σ) = 5.1 variance(μ) = 0.56 variance(σ) = 0.34 corr(μ,σ) = -0.16	mean(μ) = 25.0 mean(σ) = 1.8 variance(μ) = 0.25 variance(σ) = 0.12 corr(μ,σ) =0.47	mean(μ) = 11.6 mean(σ) = 5.1 variance(μ) = 1.0 variance(σ) = 0.85 corr(μ,σ) =-0.28
Gulf Coast (Dauphin Island, AL buoy)	mean(μ) = 14.2 mean(σ) = 2.7 variance(μ) = 1.54 variance(σ) = 0.27 corr(μ,σ) = -0.08	mean(μ) = 24.5 mean(σ) = 3.5 variance(μ) = 0.98 variance(σ) = 0.27 corr(μ,σ) =-0.55	mean(μ) = 28.9 mean(σ) = 1.5 variance(μ) = 0.11 variance(σ) = 0.11 corr(μ,σ) = -0.41	mean(μ) = 17.9 mean(σ) = 4.5 variance(μ) = 3.2 variance(σ) = 0.55 corr(μ,σ) =-0.53
Pacific Northwest (Washington State Shellfish Specialists)	mean(μ) = 8.1 mean(σ) = 1.62 variance(μ) = 0.76 variance(σ) = 0.13 corr(μ,σ) = 0.01	mean(μ) = 13.7 mean(σ) = 2.4 variance(μ) = 1.0 variance(σ) = 0.24 corr(μ,σ) =0.7	mean(μ) = 17.4 mean(σ) = 2.4 variance(μ) = 0.60 variance(σ) = 0.16 corr(μ,σ) =-0.13	mean(μ) = 10.7 mean(σ) = 2.8 variance(μ) = 0.16 variance(σ) = 0.13 corr(μ,σ) = 0.36

Source of data: National Buoy Data Center (NBDC)
http://www.seaboard.ndbc.noaa.gov/Maps/Wrldmap.shtml
and Washington State shellfish specialist N. Therien, personal communication *(131)*
NBDC measures surface water temperature (sensors are generally 1.0 to 1.5 meter deep)
[a] μ and σ denote mean and standard deviation of within region/season temperature distribution, respectively; mean(), variance(), and corr() denote the mean, variance and correlation between the parameters μ and σ across different years

In Table IV-1, μ and σ denote the population mean and standard deviation of the distribution of water temperatures within any particular year for different region and season combinations. The extent of year to year variation of these distributions is summarized by the mean and the variance of the parameters μ and σ. The mean and variance of these parameters are denoted in the table as mean(μ), variance(μ), mean(σ) and variance(σ), respectively. The correlation between μ and σ is denoted by corr(μ, σ). A positive correlation between parameters μ and σ summarizes the observation that when the mean water temperature is higher than normal the variation in temperatures from one day to the next is generally greater than that observed when the mean temperature is lower than normal. Similarly, a negative correlation summarizes the observation that temperatures are less variable when the mean water temperature is higher than normal.

For example, the NBDC buoy located at Dauphin Island, Alabama was chosen as representative of water temperatures for the Gulf Coast. Among other meteorological parameters, this buoy has recorded water and air temperatures from 1987 to the present time. In reference to Table IV-1, for the spring season (defined as April through June), the distribution of noontime water temperature was found to vary from year to year with a typical (or average) mean of 24.5^{o} C [mean(μ)]. The variance of the mean from one year to the next was 0.98^{o} C [variance(μ)] which corresponds to a standard deviation of 0.99^{o} C. Similarly, for the standard deviation of the within year temperature distributions, the central tendency across different years was an average of 3^{o} C [mean(σ)] with a variance of 0.27^{o} C [variance(σ)]. The correlation between μ and σ [corr(μ, σ)] was -0.55 indicating that the day-to-day temperatures were less variable when the overall mean temperature was higher than that of a typical year.

For the Pacific Coast there were no near-shore NBDC buoys recording water temperatures that could be considered representative of oyster growing areas. Consequently, for this region, seasonal and year-to-year variations in water temperature distributions were developed based on compiled data from WA State shellfish specialists (Washington State Department of Health) from 1988 through 1999. These water temperature data were recorded in association with collection of samples for monitoring of vibrios and fecal coliforms and are therefore directly representative of temperatures for oyster growing areas. Averages of water temperature were substituted when multiple measurements were recorded for any given day. Year-to-year variations in the water temperature distributions for the Pacific Coast were developed in the same manner as that for the other regions.

Additional sources of information concerning water temperatures (and salinity) in oyster growing areas include the EPA STORET (Storage and Retrieval of U.S. Waterways Parametric Data) database (http://www.epa.gov/OWOW/STORET/) and the National Estuarine Reserve Sites (NERR) program (http://inlet.geol.sc.edu/cdmoweb/home.html). In comparison to the NBDC sites, STORET and NERR are more specific to estuaries as opposed to open coastal waterways. Some NBDC sites such as Thomas Point Lighthouse (Chesapeake) are located within estuaries but similar sites could not be identified for the Gulf Coast and Northeast Atlantic within the NBDC database. Comparison of NERR data for Weeks Bay, AL versus that of the Dauphin Island NBDC buoy suggests that shallow water estuaries may be slightly warmer than open coastal waters but that the difference is not substantial (i.e., ~1^{o} C difference

on average). An additional consideration is the availability of enough long-term historical data to determine extent of year-to-year variation. As already indicated, data is available from most NDBC buoys from 1988 to the present. The NERR program only started data collection in 1995. Although STORET has considerable long term historical data associated with monitoring of water quality dating back to 1964, access to STORET records is not readily available at present and the data could not be accessed during the time frame of the risk assessment. Also, STORET records do not necessarily correspond to fixed locations, as is the case for NBDC and NERR.

Additional data on water temperature (and salinity) measurements specific to oyster harvesting areas were made available to the risk assessment team by State agencies in Texas, Alabama, New York, and Connecticut. Water temperatures provided were not substantially different from the NBDC data selected for each region.

Prediction of the distribution of pathogenic *V. parahaemolyticus* densities

Table IV-2 shows estimates of the percentage of total *V. parahaemolyticus* isolates that have been found to be pathogenic in several studies. The estimate based on studies by Kaysner and colleagues applies to the Pacific Northwest *(76)* with the other estimates in Table IV-2 being appropriate for all other areas of the country. The estimates suggest that the average percentage of *V. parahaemolyticus* that are pathogenic relative to total *V. parahaemolyticus*, on the West Coast is ~3% and that the average percentage pathogenic in the Gulf Coast and other areas of the country is 0.2 to 0.3%.

Table IV-2. Estimates of pathogenic V. parahaemolyticus (Vp) as a percentage of total V. parahaemolyticus

Oyster samples (12 oyster composites) containing detectable pathogenic *V. parahaemolyticus* (TDH+ or KP+)[a]			***V. parahaemolyticus* isolates that are TDH+ or KP+**			**Source**
Number oyster samples with pathogenic Vp	Number tested	Percent oyster samples with pathogenic Vp	Number isolates pathogenic	Number isolates tested	Percent isolates pathogenic	
8 TDH+	193	4.1	9 TDH+	3233	0.3%	ISSC/FDA retail study (unpublished) *(44)*
ND[b]	153 oyster, water, & sediment samples tested for KP+	ND	4 KP+	2218	0.18%	Galveston Bay , TX *(133)*
4 TDH	25	16	10 TDH	308	3.2%	Grays Harbor, WA *(73)* Puget Sound, WA *(76)*
3 TDH	96	3.1	10, 140, and 10 cfu/g in three samples	ND	0.3%	FDA study of Texas outbreak Galveston Bay, TX *(37)*

[a] KP+ - Kanagawa-positive; TDH+ - thermostable direct hemolysin-positive, a toxin produced by *Vibrio parahaemolyticus* that lyses red blood cells in Wagatsuma agar. These terms are interchangeable in defining pathogenicity of *V. parahaemolyticus*

[b] ND = not determined

There is considerable uncertainty with regard to the average percentage of total *V. parahaemolyticus* isolates that are pathogenic due to the relatively small sample sizes for estimating such a small percentage. Furthermore, this percentage is likely to vary somewhat from one year to the next. Even if an average percentage were known with certainty, this information together with the estimated distributions of total *V. parahaemolyticus* densities is not sufficient to identify the distribution of pathogenic *V. parahaemolyticus* densities. It is likely that the density of pathogenic strains is spatially and temporally clustered in the environment to some degree. The average number of isolates that are pathogenic does not identify the extent of this clustering.

To account for the probable spatial and temporal clustering of pathogenic strains relative to total *V. parahaemolyticus* densities, we have assumed a beta-binomial distribution for the number of pathogenic *V. parahaemolyticus* at the time of harvest. Under a beta-binomial distribution the percentage of total *V. parahaemolyticus* which are pathogenic varies from one sample of oysters (e.g. 12 oyster composite) to the next. Given the occurrence of outbreaks this appears to be a reasonable assumption but cannot be validated directly since extensive quantitative surveys of pathogenic *V. parahaemolyticus* densities are not available. Specifically, based on the number of total *V. parahaemolyticus* (Vp_{total}), within a given composite, the number of pathogenic (Vp_{path}) present is assumed to be distributed as a binomial random variable with Vp_{total} trials (size parameter) and a probability of success (p) distributed as a beta random variable. The distribution of the probability parameter p is called a mixing distribution and the variation of this parameter across composites of oysters induces a clustering of pathogenic strains relative to total *V. parahaemolyticus*.

Formally this beta-binomial model is expressed as:

$$Vp_{path} \mid (Vp_{total} = n) \quad \sim \quad B(n, p) \bigwedge_{p} Beta(\alpha, \beta)$$

The notation here indicates that the distribution of the number of pathogenic *V. parahaemolyticus* present is conditional on the number of total *V. parahaemolyticus* present (n). The mean and variance of this conditional distribution are:

$$E[Vp_{path} \mid Vp_{total} = n] = \frac{\alpha}{\alpha + \beta} * n$$

$$Var[Vp_{path} \mid Vp_{total} = n] = n * \left[\frac{\alpha * \beta}{(\alpha + \beta)^2} \left(1 + \frac{1}{\alpha + \beta + 1} (n - 1) \right) \right]$$

$$= n * \left[\frac{\alpha * \beta}{(\alpha + \beta)^2} \left(1 + \phi * (n - 1) \right) \right]$$

where E[•] and Var[•] denote the mean and variance, respectively. The parameter ϕ is called the overdispersion parameter. The parameters α and β of mixing distribution in the beta-binomial can expressed in terms of the average percentage of isolates which are pathogenic (P), which is the mean of the mixing distribution, and the dispersion parameter ϕ:

$$\alpha = \frac{P * (1 - \phi)}{\phi}$$

$$\beta = \frac{(1 - P) * (1 - \phi)}{\phi}$$

From Table IV-2, best estimates of the parameter *P* are 0.03 for the West Coast and 0.002 for other regions of the country. The information is more limited with respect to the value of the shape parameter ϕ. This parameter pertains to the variation of frequency of pathogenic *V. parahaemolyticus* across different oyster samples or composites. Based on the data on frequency of pathogenic isolates, Bayes estimates of the parameters α and β are:

$$\hat{\alpha} = r + 1$$
$$\hat{\beta} = n - r + 1$$

where r is the number of pathogenic isolates and n is the total number of isolates. These estimates differ for the West Coast versus other regions of the country in the same manner as does average percentage pathogenic (*P*). An estimate of the dispersion parameter is:

$$\hat{\phi} = \frac{1}{\hat{\alpha} + \hat{\beta} + 1} = \frac{1}{n + 2}$$

Based on the data shown in Table IV-2, estimates of the dispersion parameter are 0.0032 for the West Coast and 0.00045 for the other regions of the country.

To the extent that the average percentage of isolates that are pathogenic is uncertain, and may vary from year to year, *P* was evaluated as an uncertainty parameter in the Monte Carlo simulations. The uncertainty was modeled as a triangle distribution with a different mean and range for the Pacific Northwest than for other regions of the country. For the Pacific Northwest the average percentage pathogenic was estimated to be 3% and the minimum and maximum of the distribution was taken to be 2% and 4%, respectively. For all other regions of the country the average percentage pathogenic was estimated to be 0.2% and the corresponding minimum and maximum of the distribution was 0.1% and 0.3%, respectively. Uncertainty with regard to the shape parameter ϕ was not evaluated.

Overall, the Monte Carlo simulation of the distribution of pathogenic *V. parahaemolyticus* present in oysters was preformed as follows. For each region and season the mean and standard deviation of water temperature distributions were sampled based on the bivariate normal distributions given in table IV-1. Each random sample from these distributions represents a distribution of water temperature (i.e., for different years). Given a water temperature distribution, the distribution of total *V. parahaemolyticus* densities in composites of 12 oysters at harvest was simulated by (a) sampling from the distribution of water temperature; (b) using the regression relationship to calculate a mean density corresponding to each sampled water temperature; and (c) perturbing the calculated means by a random normal deviate corresponding to the estimate of population variation of the densities. The distribution of pathogenic *V. parahaemolyticus* densities was derived from that of total *V. parahaemolyticus* assuming a beta-binomial model for the extent of clustering of pathogenic relative to total counts. Multiple simulations were run with different values of average percentage of isolates pathogenic in order to evaluate the uncertainty with regard to this parameter.

Post Harvest Module

The Post Harvest Module describes the effects of typical industry practices, including transportation, handling and processing, distribution, storage, and retail, from harvest to consumption, on *V. parahaemolyticus* densities in oysters harvested from various locations and seasons. Factors considered as possible influences on the levels of pathogenic *V. parahaemolyticus* at consumption include: ambient air temperatures at time of harvest; time from harvest until the oysters are placed under refrigeration; time it takes the oysters to cool once under refrigeration, and length of refrigeration time until consumption. This module also describes possible intervention strategies, such as mild heat treatment, freezing, hydrostatic pressure, depuration, and relaying, which could reduce *V. parahaemolyticus* densities.

Although the ecology of *V. parahaemolyticus* has been studied extensively *(69, 70)*, little is known about the growth and survival of *V. parahaemolyticus* in shellstock oysters *(30)* or the effectiveness of mitigations aimed at reducing *V. parahaemolyticus* levels. The effects of post harvest storage on *V. vulnificus* growth in oyster shellstock *(26, 27)*, and the effectiveness of various mitigation strategies for reducing *V. vulnificus* have been studied more extensively *(29, 42, 99, 113, 122)*. Similar approaches are currently under investigation for *V. parahaemolyticus* and some preliminary data are included in this section.

The National Shellfish Sanitation Program (NSSP) time/temperature matrix for control of *V. vulnificus* requires oyster harvesters from any state, which previously had two or more confirmed cases of *V. vulnificus* to refrigerate oysters within 10 hours (h) after harvest during summer months, depending on water temperature. This provides approximately 10-fold reduction in *V. parahaemolyticus* growth relative to 20 h required in other months and on other coasts.

Mitigation Strategies

Proposed mitigation processes such as mild heat and freezing, which have been shown to be effective in reducing *V. vulnificus* levels, would probably have a similar effect on *V. parahaemolyticus* but only limited data is currently available *(29)*. Other possible strategies include irradiation, high pressure, depuration and relaying. However, we have no relevant data on their effectiveness on *V. parahaemolyticus* in shellstock oysters, and had to rely on data from studies on *V. vulnificus*.

Reducing time to refrigeration

- It has been shown from the literature that a reduction in the extent of growth of 0 to 10,000-fold in *V. parahaemolyticus* densities could be achieved depending on the initial *V. parahaemolyticus* levels, ambient air temperature and time to refrigeration *(30, 51, 66, 67)*.

Mild heat treatment

A 6-log reduction of natural *V. vulnificus* population was achieved by heating shucked oysters for 5 min at 50° C *(29).* Similar heat sensitivity was observed between *V. parahaemolyticus* and *V. vulnificus (51).* Assuming that *V. parahaemolyticus* responds similarly to heat as. *V. vulnificus,* a 4.5 to 6-log (1,000,000-fold) reduction of *V. parahaemolyticus* densities could be expected by treating oysters for 5 min at 50° C.

Freezing treatment

A 1973 study reported a two-stage mortality for *V. parahaemolyticus* with an initial stage of cold shock followed by a second stage related to the frozen storage conditions *(66).* Estimates of effect of cold shock and frozen storage conditions were obtained by regression analysis of the observed data. Based on the analysis, freezing combined with frozen storage for 30 days at –30° C and –15° C is projected to result in a 1.2 and 1.6 $\log_{10}$ reduction of *V. parahaemolyticus* numbers in oysters, respectively. A similar decline (2 to 3 logs) of *V. parahaemolyticus* (natural population and dosed with pathogenic O3:K6 serotype) was observed in oysters frozen 35 days at –20° C *(25).* Freezing combined with frozen storage for 30 days would be expected to produce approximately a 2 log reduction of pathogenic *V. parahaemolyticus.* Both pathogenic strains (TDH^+) and non pathogenic (TDH^-) *V. parahaemolyticus* respond similarly to freezing *(25).*

Depuration

In the United States, depuration is conducted exclusively with UV light disinfection *(113).* There is a broad spectrum of conditions under which shellfish are depurated. Optimal times, temperatures and salinities for effective depuration vary among shellfish species. Published literature has shown that depuration appears to have no significant effect on decreasing the level of *Vibrio* spp. in naturally infected oysters or clams, and these microbes may even multiply in depurating shellfish, tank water, and plumbing systems *(42, 53).* A 1 log reduction of *V. parahaemolyticus* was observed in the hardshell clam, *Mercinaria mercinaria,* after 72 h of depuration at room temperature *(53),* and >2 log reduction at 15° C *(52).* Son and Fleet *(122)* observed a 5 log reduction in lab-infected oysters (from $9x10^7$ to $8x10^2$ in 72 h). Eyles and Davey *(42)* showed no difference ($p<0.1$) before and after depuration in naturally infected oysters.

Relaying

Relaying is the process by which shellfish are cleansed by transferring them to clean shellfish growing areas. There is little data available on this approach, which is also problematic as *V. parahaemolyticus* is ubiquitous in estuarine environments. Son and Fleet *(122)* demonstrated a decrease from 18 *V. parahaemolyticus*/g to < 5 *V. parahaemolyticus*/g after 6 days.

Modeling of the Post Harvest Module

The purpose of modeling the Post Harvest Module is to simulate the effects of typical industry practices on the levels of *V. parahaemolyticus* in oysters from harvest to consumption for various locations and seasons. The module also simulates the effect of intervention strategies. The input to the module is the regional and seasonal distributions of total and pathogenic *V. parahaemolyticus* at harvest. The output of the module is a series of predicted distributions of the total and pathogenic densities at time of consumption. Figure IV-6 represents a diagrammatic representation of the parameters modeled in this section. The baseline prediction is the distribution of density of *V. parahaemolyticus* (in 12 oyster composites), assuming current industry practices and no intervention.

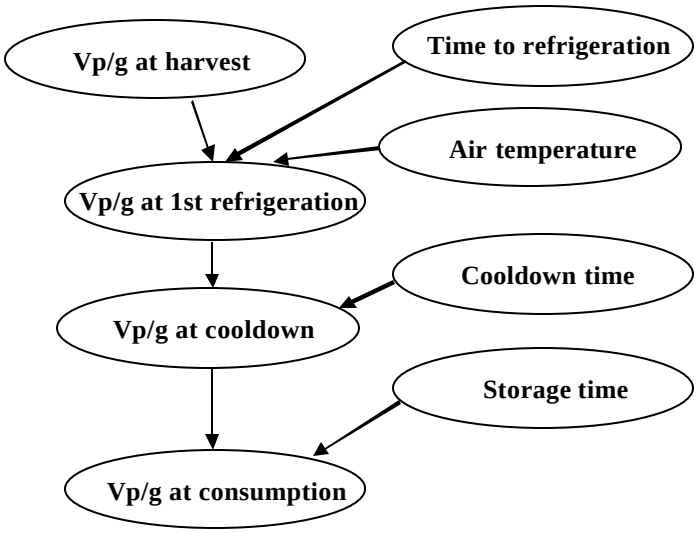

Figure IV-6. Schematic depiction of the Post Harvest Module of the V. parahaemolyticus (Vp) risk assessment model.

The principle assumption used to develop the relationships between densities at harvest and densities at time of consumption is that the growth and survival of pathogenic *V. parahaemolyticus* is the same as total *V. parahaemolyticus*. Although no definitive studies of the growth characteristics of pathogenic *V. parahaemolyticus* are available, preliminary data suggest that there is little difference between growth characteristics of pathogenic versus nonpathogenic strains *(37).* Furthermore, observation of the growth of total *V. parahaemolyticus* in oysters is limited to only one temperature (26^{o} C). To bridge this data gap we have used a model of *V. parahaemolyticus* growth in broth developed by Miles *et al. (95).* The predictions of this model have been adjusted to predict the growth rate in oysters, which is less than that of broth model systems possibly due to the influence of competing microflora.

Growth of *V. parahaemolyticus* from harvest to first refrigeration

The extent of growth that occurs during the period of time from harvest until the time that oysters are first placed under refrigeration is determined by three factors: (a) the growth rate of *V. parahaemolyticus* as a function of temperature; (b) the temperature of oyster meat following harvest and (c) the length of time held unrefrigerated.

Growth Rate Model

Miles *et al. (95)* modeled the growth rate of *V. parahaemolyticus* based on studies of four strains at different temperatures and water activity, which is a measure of the availability of free water in the broth model system. Worst case estimates of growth were obtained based on the fastest growing of the four strains studied. For each combination of temperature and water activity, the extent of bacterial growth observed was modeled using the Gompertz function. This is a sigmoid growth curve with a growth rate (slope) monotonically increasing up to a maximum and then falling to zero as the bacterial population reaches a steady-state. The maximal rate of growth (μ_m) is the most relevant summary of the fit because the growth rate approaches the maximal growth rate rapidly and does not decline significantly until steady-state is reached.

A secondary model was used to estimate the effect of environmental parameters on the maximal growth rate. This model was assumed to be of the square root type:

$$\sqrt{m_m} = \frac{b*(T-T_{\min})*\left[\{1-\exp(c*(T-T_{\max}))\}*\sqrt{(a_w-a_{w,mix})*[1-\exp(d*(a_w-a_{w,\max}))]}\right]}{\sqrt{\ln(10)}}$$

where

μ_m = maximal growth rate ($\log_{10}$ per minute)
a_w = water activity
T = temperature (in degree Kelvin)

Based on the data from the fastest growing strain the estimates of the parameters were:

b	=	0.0356
c	=	0.34
T_{min}	=	278.5
T_{max}	=	319.6
$a_{w,min}$	=	0.921
$a_{w,max}$	=	0.998
d	=	263.64

The parameters T_{min}, T_{max}, $a_{w,min}$, and $a_{w,max}$ denote the range of temperatures and water activity over which growth can occur. The authors validated their model by comparison of model predictions with observed rates in eight other studies of growth in broth model systems obtained from the literature.

A plot of the resulting model prediction for μ_m as a function of either temperature or water activity is a unimodal function with a maximum value and zero growth rate outside of the predicted range of temperatures and water activity favorable for growth. To use this equation as a prediction of growth rate in oysters we assumed that water activity of oysters does not vary substantially and have fixed this parameter to the optimal value of 0.985 predicted for the broth model system. At this water activity, the predicted growth rate in broth at 26° C is 0.84 $\log_{10}$ per hour which is approximately a 7-fold increase in density per hour. This is four times greater than the rate of growth observed for *V. parahaemolyticus* in oysters held at 26° C *(51)*.

Based on this observation, our best prediction of the growth rate in oysters at temperatures other than 26° C was obtained by dividing the predicted rate for broth model by a factor of four. This assumes that the growth rate in oysters is a constant fraction of the growth rate in broth at all temperatures. We have evaluated the influence of this assumption in the risk assessment by considering this factor as an uncertainty parameter varying according to a triangle distribution in the range of 2 to 8 with a mean of 4. This evaluates the sensitivity of our conclusions to the magnitude of the relative growth rate in oysters versus broth model but does not fully address the uncertainty in so far as it is conceivable that the relative growth rate could be temperature dependant.

The use of the Gompertz function by Miles *et al. (95)* to model bacterial growth in broth is appropriate. After transfer of an inoculum to different medium or environmental conditions there is a demonstrable lag phase during which the bacterial population adapts to different environmental conditions and growth is suboptimal *(24)*. However, the Gompertz is not an appropriate model for growth of *V. parahaemolyticus* in oysters after harvesting, as changes in environment are typically gradual and do not arrest the growth rate and induce a lag phase. Consequently, for oysters, the extent of growth occurring over time at a given average temperature and predicted maximal growth rate is assumed to follow a simple three-phase loglinear model with no lag phase *(19)*. This model is of the form:

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

where $N(t)$ refers to the bacterial density at a given time (t) post harvest, A is the logarithm of the maximum attainable density of *V. parahaemolyticus* in oysters, and the parameter μ_m is a function of ambient temperature as described above. At 26° C, the density of *V. parahaemolyticus* in oysters was observed to approach a plateau of approximately 6.0 $\log_{10}$ per gram after 24 hours *(51)*. We have assumed this value for the maximal density (A) at all temperatures. Figure IV-7 shows predictions of the $\log_{10}$ increase in *V. parahaemolyticus* density from an initial level of 1,000/g as a function of time for three ambient temperatures (20, 26 and 32° C).

Ideally, the average temperature used to determine the parameter μ_m in the above equation is the temperature of oyster meat of shellstock. Clearly the temperature of oyster meat depends on the temperature of both the air and water at the time of harvest. Temperature of

the oyster meat after harvest is expected to gradually equilibrate with the temperature of the air and may be modified somewhat by evaporative cooling and the extent to which oysters are properly shaded from direct sunlight aboard ship. In the absence of information to the contrary, we have assumed that the temperature of oyster meat equilibrates rapidly with that of the ambient air and have therefore used air temperature as a surrogate for oyster meat temperature. Ambient air temperature data recorded at noon from the near-shore NBDC buoys representative of various coastal regions were used for this purpose.

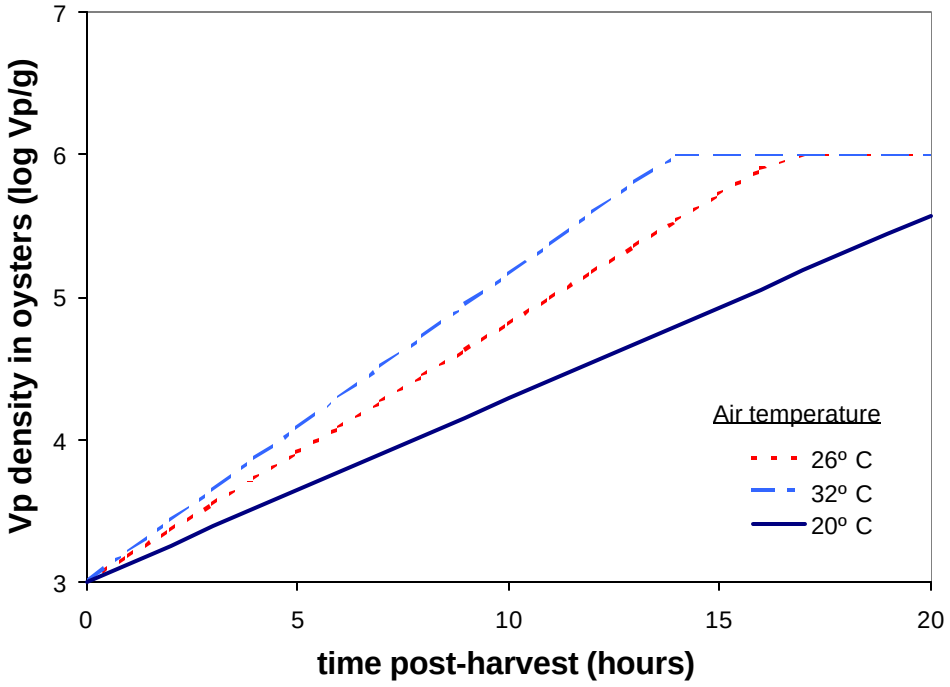

Figure IV-7. Predicted loglinear growth of *V. parahaemolyticus* (Vp) from initial density of 1,000 (3 $\log_{10}$) Vp/g as a function of ambient air temperature.

Distribution of ambient air temperature

Examination of water and air temperatures obtained from the NOAA/NBDC database showed a strong correlation between water and air temperature. This correlation has been incorporated into the risk simulation by modeling the distribution of the difference in water versus air temperatures based on the normal distribution within any given region and 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.

In the process of simulating the distribution of total and pathogenic *V. parahaemolyticus* at harvest by the Monte Carlo method, the water temperature associated with any given outcome is retained. A corresponding air temperature is predicted by 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 difference in air versus water temperature were obtained by pooling the data available for each near-shore buoy across all available years. The mean and variance of these distributions are shown in Table IV-3.

Table IV-3. Means and standard deviations of the distribution of the difference between recorded air and water temperatures at midday (° C)

Region	Mean (standard deviation) Distribution Differences between Air and Water Temperature			
	Winter (Jan-March)	**Spring** (April-June)	**Summer** (July-Sept)	**Fall** (Oct-Dec)
Northeast Atlantic (Ambrose buoy, NY harbor)	-2.6 (5.0)	2.2 (3.2)	0.52 (2.7)	-3.2 (4.2)
Mid-Atlantic (Thomas Point Lighthouse buoy, Chesapeake Bay, MD)	-0.25 (4.0)	0.54 (2.9)	-1.4 (2.1)	-2.1 (3.1)
Gulf Coast (Dauphin Island, AL buoy)	-1.07 (3.3)	-1.24 (1.63)	-1.66 (1.33)	-1.62 (3.3)
Pacific Northwest (based on 3 years of data from NOAA buoy on north end of Puget Sound, WA)	-1.6 (1.8)	1.3 (1.3)	1.3 (1.5)	-0.8 (2.0)

Source of data: http://www.seaboard.nbdc.noaa.gov/Maps/Wrldmap.shtml

Distribution of time oysters are left unrefrigerated

The distribution of the length of time that oysters are held unrefrigerated was developed by using the distribution of duration of daily oyster harvesting operations (i.e., length of working day). The distribution of length of time oysters are left unrefrigerated is derived by assuming that oysters are harvested uniformly from the start of the harvest up to one hour prior to conclusion of the harvesting operation when oysters are landed and placed in cold storage.

Table IV-4 shows the minimum, maximum and mean duration of oyster harvesting that we have projected for the different regions and seasons. In the risk simulation, we have used Beta-PERT distributions based on these parameters to simulate the variation in the duration of harvesting. A Beta-PERT distribution is a translated and scaled Beta distribution with specified moments. It is commonly used for the purpose of simulating parameter variation within a defined range in Monte Carlo simulations. Figure IV-8 shows the probability density of the Beta-PERT distribution with minimum of 2, maximum of 11 and mean of 8 hours.

Table IV-4. Minimum, maximum and mean duration of oyster harvest (length of harvesting operation) for different regions and seasons

Location	Duration of Harvest (hours)			
	Winter (Jan-March)	**Spring** (April-June)	**Summer** (July-Sept)	**Fall** (Oct-Dec)
Northeast Atlantic (assumed same as pre-NSSP Control plan in Gulf- TX *(64)*)	max = 11 min = 2 mean = 8	max = 11 min = 2 mean = 8	max = 11 min = 2 mean = 8	max = 11 min = 2 mean = 8
Mid-Atlantic (assumed same as pre-NSSP Control plan in Gulf- TX *(64)*)	max = 11 min = 2 mean = 8	max = 11 min = 2 mean = 8	max = 11 min = 2 mean = 8	max = 11 min = 2 mean = 8
Gulf Coast - LA (50% of harvest) (pre-NSSP Control plan in LA in winter; ICP otherwise *(64)*)	max = 13 min = 7 mean = 12	max = 11 min = 5 mean = 9	max = 11 min = 5 mean = 9	max = 13 min = 7 mean = 12
Gulf Coast - FL, AL, TX (50% of harvest) (assumed same as pre-NSSP Control plan in Gulf- TX in winter, NSSP Control otherwise *(64)*)	max = 11 min = 2 mean = 8	max = 10 min = 3 mean = 7	max = 10 min = 3 mean = 7	max = 10 min = 3 mean = 7
Pacific Northwest *(139)*	max = 4 min = 1 mean = 3	max = 4 min = 1 mean = 3	max = 4 min = 1 mean = 3	max = 4 min = 1 mean = 3

Source of data: ISSC & FDA (ed.) 1997 National Shellfish *(64)*
Washington State Shellfish experts and Washington State Department of Health *(139)*

The parameters for these distributions were developed and based on a 1997 GCSL survey that included dealer reported statistics on the length of harvest *(28).* The study was conducted in several Gulf Coast states during the fall of two successive years; one season prior to initiation of the NSSP time to refrigeration requirements (for states whose product has been confirmed as the source of two or more *V. vulnificus* illnesses), and then the following year after implementation. Duration of harvest was longer in Louisiana than in Florida and Texas, during both years. This

probably reflects more remote oyster harvesting areas in Louisiana. The practices of Florida and Texas were considered to be representative of other regions, and in the absence of conflicting information, the longer times were assumed for the other regions throughout the year.

For the Gulf Coast States, we assumed that current harvesting duration is limited in the spring, summer and fall due to the NSSP time to refrigeration requirements and that duration of harvest is generally longer in the winter when cooler water conditions prevail. Louisiana, representing roughly half of the Gulf Coast harvest was treated separately due to the longer duration of harvest year round. The distribution of harvest duration for the West Coast was not based upon the GCSL dealer survey in so far as oysters are generally harvested during intertidal periods and the length of time held unrefrigerated is substantially less. The Pacific Coast Shellfish Growers Association (PCSGA) stated that Pacific oysters are placed under refrigeration within four hours and this time is being assumed as the maximum for the Pacific Coast in the absence of survey data.

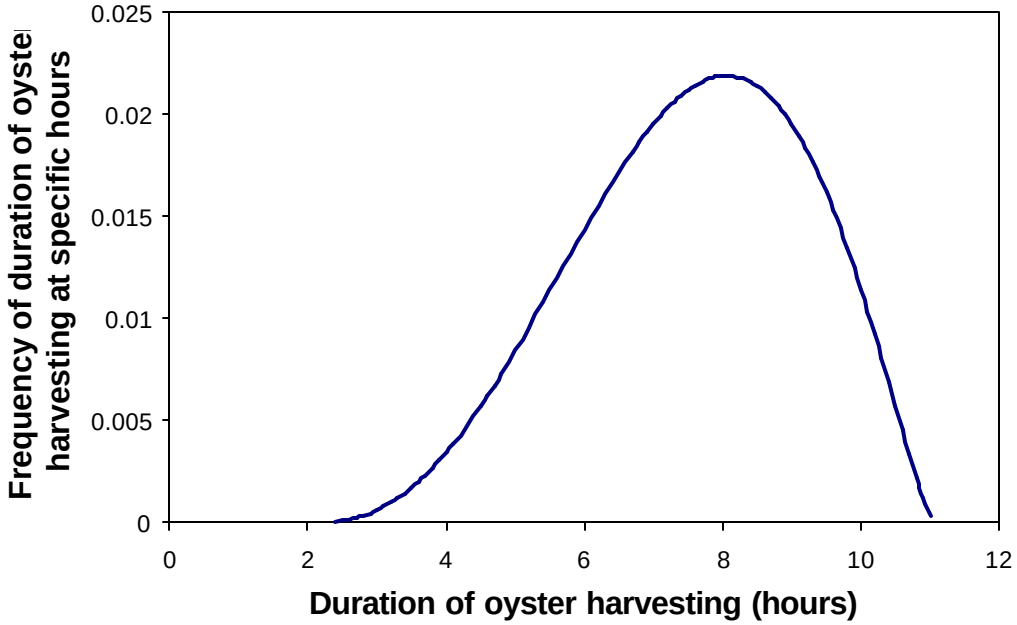

Figure IV-8. Beta-PERT probability density distribution for the duration of harvesting operations during the winter season (Mid-Atlantic, Northeast Atlantic, Gulf Coast, excluding Louisiana) *(44).*

As indicated, harvesting of oysters was assumed to occur uniformly from start of harvest, up to one hour prior to end of harvest operation. The distribution of the duration of time oysters were held unrefrigerated, was simulated by first sampling from the distribution for duration of harvest operation and then sampling from a uniform distribution with a minimum of one hour and maximum corresponding to the randomly selected duration of harvest. Oysters are harvested at different times during the length of harvesting operations. Consequently the mean time that oysters remain unrefrigerated is much less than the maximum length of duration of harvesting might suggest.

Overall, the extent of growth occurring prior to time of first refrigeration (i.e., the time at which oysters are first placed in refrigerated storage) was simulated by: (a) sampling air temperature corresponding to the water temperature at harvest; (b) sampling duration of harvest; (c) sampling the length of time unrefrigerated given a particular duration of harvest; and then (d) calculating the extent of growth expected for the given duration of time unrefrigerated.

Excess growth of *V. parahaemolyticus* during cooldown time

V. parahaemolyticus will continue to grow in oysters after they are placed under refrigeration until the temperature of the oyster tissues falls below a certain threshold (e.g. 10° C). The time it takes for oysters to cool once under refrigeration is assumed to be quite variable depending on efficiency of the cooler, quantity of oysters to be cooled and their arrangement in the cooler. Data on cooling rates of commercial oyster shellstock could not be located. 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 *(37)*. However, 24 oysters in an uninsulated plastic container required approximately 7 hours to drop from 26° C to 3° C. These data suggest considerable uncertainty for cooling times after oysters are refrigerated and it was concluded that a rectangular distribution between 1 and 10 hours would be appropriate to describe the current state of knowledge.

As oysters cool down to storage temperatures it is reasonable to expect that the growth rate of *V. parahaemolyticus* slows with the declining temperature of the oyster tissue. At the start of the cooldown period, when oysters are first placed under refrigeration, the growth rate is still equal to the initial rate as determined by ambient air temperature. At the end of the cooldown period, when oysters have reached storage temperatures, we assume that there is no further growth and that densities will decline slowly thereafter. Implicitly, this assumes that there is no appreciable temperature abuse after oysters have been placed in cold storage. The rate at which oysters cooldown during cold storage is not known. Therefore, in the absence of conflicting information, we have assumed that during the period of cooldown, the growth rate of *V. parahaemolyticus* drops uniformly down to zero.

A discrete approximation of the extent of growth that may occur during cooldown was simulated by first sampling from a discrete random uniform distribution between 1 and 10 hours (duration of cooldown). The extent of growth during each hour of the cooldown period was then approximated by an average growth rate during that hour times a duration of one hour. The average growth rates were dependant upon the growth rate of *V. parahaemolyticus* in oysters left unrefrigerated (i.e., as determined by the ambient air temperature for a given oyster

lot) and the duration of cooldown. Total excess growth was the sum of these values over the cooldown period subject to the restriction that the maximum density of 6.0 $\log_{10}$ per gram could not be exceeded. These calculations are illustrated in the Table IV-5, where, for example, it takes k hours for a particular oyster lot to reach cooler temperature.

Table IV-5. Discrete approximation of variation in the growth rate of *V. parahaemolyticus* during a cooldown period of k hours

Hour of the cooldown period	Average growth rate ($\log_{10}$/hr) during the hour of cooldown
1	$\frac{(k+1)-1}{k} m_m$
2	$\frac{(k+1)-2}{k} m_m$
3	$\frac{(k+1)-3}{k} m_m$
...	...
k	$\frac{(k+1)-k}{k} m_m$
k+1	0

Total excess growth is the sum of the growth over the k hours:

$$\sum_{i=1}^{k} m_m * \frac{(k+1)-i}{k} = m_m * \left[(k+1) - \frac{1}{k} \sum_{i=1}^{k} i \right]$$

$$= m_m * \left[(k+1) - \frac{k+1}{2} \right]$$

$$= m_m * \frac{k+1}{2}$$

Since the cooldown time k is a random variable with a mean of 5.5 hours, the average extent of growth is $3.25*\mu_m$, where μ_m is the maximal growth rate determined by ambient air temperature at time of harvest. Thus, for an initial growth rate of 0.19 $\log_{10}$ per hour (i.e., at 26° C), the average growth occurring during cooldown is approximately 0.6 $\log_{10}$.

Die-off of *V. parahaemolyticus* during cold storage

Gooch *et al. (51)* showed that in oysters, *V. parahaemolyticus* declined 0.003 $\log_{10}$ per hour when stored 14-17 days at 3° C. This die-off rate was assumed to be typical of all refrigerated oysters. Error may be introduced because commercial oysters are typically stored at higher temperatures (5-10° C). Die-off may have been overestimated because chill-stressed *V. parahaemolyticus* may not be recovered by the methods used in the study. One of the enumeration methods employed a repair step in a medium containing magnesium, which has been shown to increase recovery of chill-stressed cells. This method did not give higher *V. parahaemolyticus* counts after refrigeration than did the other methods that were used to calculate die-off. Therefore, the effect of chill-stress on die-off rate was assumed to be negligible.

Data from the ISSC/FDA retail study for the time between harvest and sample collection were assumed to be a reliable estimate for the length of refrigeration time (time between refrigeration and consumption) *(28).* Summary statistics on the storage time for samples obtained during the study are shown in Table IV-6. A small degree of error may be introduced by assuming that these data are representative of storage time in so far as samples were generally collected on Monday or Tuesday and most servings are consumed in restaurants on weekends. Since this was a year long nationwide survey, the mean of 7.7 days and range of 1-21 days was assumed to be representative of all seasons and regions. In the simulation, we used a Beta-PERT distribution based on the overall mean, minimum, maximum and mode in order to obtain a smooth representation of the variation in the duration of storage time.

Table IV-6. Summary statistics of the distribution of storage times (time under refrigeration in days) of oysters samples obtained during the ISSC/FDA retail study

Storage Time	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
Mode	6	5	6

Source of data: *(44)*

The predicted densities of *V. parahaemolyticus* at time of consumption were therefore simulated by randomly sampling from the distribution of storage times and multiplying by a die-off rate of 0.003 $\log_{10}$ per hour. The resulting distribution was then subtracted from the predicted distribution of *V. parahaemolyticus* densities in oysters initially reaching cooler (no growth) temperatures.

Mitigation Strategies

The effects of three possible post harvest mitigations were evaluated in the Monte Carlo simulations: (a) reduction of time to refrigeration (rapid cooling); (b) heat treatment and (c) freezing/cold storage.

The mitigation of reduction in time to refrigeration was modeled by assuming that oysters would be cooled to no growth temperatures immediately following harvest. Immediate cooling would involve icing or otherwise refrigerating oyster shellstock aboard ship while oyster harvesting operations continued. Assuming that this mitigation practice was followed without exception, post harvest growth of *V. parahaemolyticus* in oysters would occur only during the period of cooldown required for the oyster meat to reach no growth temperatures. In the simulation this is accomplished by assuming that the time unrefrigerated is zero (i.e., a degenerate distribution or constant). However, some growth is still projected to occur during cooldown as described above.

The effects of heat treatment and that of freezing/cold storage were evaluated by adjusting the simulated output of the baseline simulation (no mitigation) downward by factors of 4.5 $\log_{10}$ (the lowest level which caused a substantial reduction in illness after mild heat treatment) and 2 $\log_{10}$, respectively. Thus, random sequences of values for total and pathogenic densities produced in the course of Monte Carlo simulation were divided by 31,623 and 100, respectively. The implicit assumption here is that the effect of treatment on $\log_{10}$ *V. parahaemolyticus* densities is uniform with no induced change in the variance of $\log_{10}$ densities. The effects of these mitigations on the probability of illness are shown in the Risk Characterization Section.

Public Health Module

The Public Health Module estimates the distribution of the probable number of illness which may be expected to occur within any given region and season based on the predicted distribution of pathogenic *V. parahaemolyticus* densities at time of consumption, and the resulting effects on members of the public eating these oysters. Factors taken into account include the number of *V. parahaemolyticus* infections, the level of pathogenic *V. parahaemolyticus* at consumption, the probability of *V. parahaemolyticus* infection at different dose levels, and the number of diarrheal cases as opposed to more serious outcomes such as septicemia.

Food surveys and oyster landing statistics provide a basis for estimating extent of exposure in the population. Dose-response relationships can be developed from epidemiological investigations of outbreaks and sporadic case series, human feeding trials or animal models of *V. parahaemolyticus* and related (surrogate) pathogens. The relevant parameters relating to extent of exposure and dose-response relationship are summarized under three sections: epidemiology, consumption, and dose-response (hazard characterization).

Epidemiology

Gastroenteritis due to *V. parahaemolyticus* infection is usually a self-limiting illness of moderate severity and short duration *(11, 12, 89)*. However, severe cases requiring hospitalization have been reported. A summary of clinical features associated with *V. parahaemolyticus* gastroenteritis infection is presented in Table IV-7 *(12, 89)*. Symptoms include explosive watery diarrhea, nausea, vomiting, abdominal cramps, and less frequently headache, fever and chills. On rare occasions, septicemia, an illness characterized by fever or hypotension and the isolation of the microorganism from the blood, can occur. In these cases, subsequent symptoms can include swollen, painful extremities with hemorrhagic bullae *(57, 83)*. Duration of illness can range from 2 hours to 10 days *(12, 13)*.

Table IV-7. Clinical symptoms associated with gastroenteritis caused by *V. parahaemolyticus*

Symptoms	Incidence of symptoms	
	Median	Range
Diarrhea	98%	80 to 100%
Abdominal cramps	82%	68 to 100%
Nausea	71%	40 to 100%
Vomiting	52%	17 to 79%
Headache	42%	13 to 56%
Fever	27%	21 to 33%
Chills	24%	4 to 56%

Source of data: *(12, 89)*

In Japan, after a decrease in *V. parahaemolyticus* infections, the incidence started to rise again in 1994 *(9)*. There were 292 incidents of *V. parahaemolyticus* involving 5,241 cases in 1996. In 1997, the incidence increased to 568, with 6,786 cases, and in 1998, there were 850 incidents, second only to *Salmonella* infections, but involving more cases than *Salmonella (9)*. In the United States, outbreaks involving over 700 cases in the Gulf Coast, the Northeast, and the Pacific Northwest, in 1997 and 1998, were caused by consumption of raw molluscan shellfish, predominantly oysters, harboring pathogenic *V. parahaemolyticus (7, 17, 18)*. During the outbreaks, certain serotypes, linked to the consumption of raw molluscan shellfish, particularly oysters, were identified as important emerging pathogens.

Outbreaks

An outbreak is defined as the occurrence of 2 or more cases of a similar illness resulting from the ingestion of a common food. The incubation period ranges from 12-96 hours with a median of approximately 15-24 hours. The number of raw oysters consumed ranges from 1-109 (median of 12); however, the duration of consumption is not known. The typical prevalence of symptoms for cases with gastroenteritis parallels those that were identified during the Pacific Northwest outbreak of 1997. These symptoms include diarrhea (99%), abdominal cramps (88%), nausea (52%), vomiting (39%), fever (33%), and bloody diarrhea (12%). The first

confirmed case of foodborne illness-associated *V. parahaemolyticus* infection in the United States occurred in Maryland in 1971 with an outbreak caused by contaminated steamed crabs *(32)*. Between 1973 and 1998, forty outbreaks were reported to the CDC from 15 states and the Guam Territories *(34)*. These outbreaks were all associated with seafood or cross-contamination with raw or undercooked seafood. In 1997, *V. parahaemolyticus* infection was confirmed in 209 persons who consumed raw oysters harvested from California, Oregon and Washington in the United States and from British Columbia in Canada *(22)*. Prior to this outbreak, the last large outbreak of *V. parahaemolyticus* infections in North America occurred in 1981 with 6 culture-confirmed cases *(107)*. In 1998, the largest outbreak in the United States occurred in Texas in which a total of 416 *V. parahaemolyticus* infections were associated with consuming raw oysters harvested from Galveston Bay *(34)*. The first reported outbreak associated with raw shellfish harvested in New York occurred in 1998 as well, involving 23 culture-confirmed cases *(21)*. For these recent outbreaks, the dates of onset of illness ranged from May-December with a peak in July-August. Although *V. parahaemolyticus* outbreaks are less frequent in occurrence, sporadic cases are not infrequent, as further described below.

Case Reports

Several case reports have been published that outline clinical presentations and outcomes of patients with *V. parahaemolyticus*. One such case report describes a 35-year-old woman who sought medical attention for abdominal pain after she had consumed raw fish *(127)*. She presented with gastrointestinal symptoms, redness on lower extremities, fever, polyarthritis and weakness. *V. parahaemolyticus* was isolated in the stool culture. She was diagnosed as having reactive arthritis induced by *V. parahaemolyticus* infection. Another clinical case report describes a 31-year-old female with a history of alcohol abuse, hepatitis C virus infection, and cirrhosis *(54)*. She presented with diarrhea, weakness, leg pain, and urine retention. The patient had ingested raw oysters and steamed shrimp 72 hours prior to admission. *V. parahaemolyticus* was isolated from blood samples. The patient developed cardiac arrest and died six days after presentation.

A suspected case of a laboratory-associated infection was reported in 1972 *(117)*. One day prior to the development of diarrheal disease the laboratory worker had been handling *V. parahaemolyticus* strains for the first time. The illness was associated with severe upper abdominal pain, bloody stools, nausea and fever. Weakness and abdominal discomfort continued for 2 days beyond the onset of illness. No other source of *V. parahaemolyticus* could be identified, and it was believed that the infection was caused by a relatively small inoculum *(117)*.

Case Series

A case series is a study of sporadic cases over a period of time**.** Sporadic cases of *V. parahaemolyticus* infections are commonly reported by many states but are primarily reported by Gulf Coast states. Most *V. parahaemolyticus* infections present clinically as gastroenteritis, which has a low case fatality rate. Life threatening septicemia can occur, especially in patients with underlying medical conditions. The case series has a range of infection throughout the year, with a peak in September to October. A case series of *Vibrio* infections related to raw

oyster consumption was reported in Florida from 1981-1994 *(57).* Culture-confirmed case reports of *Vibrio* infections, reported to the Florida Department of Health and Rehabilitation Services, were investigated to determine the epidemiology of raw oyster-associated *Vibrio* infections. Clinical and epidemiological information from patients was compiled using standardized *Vibrio* illness case report forms. Oyster-associated *Vibrio* infection was defined as a history of raw oyster consumption in the week prior to onset of gastroenteritis or septicemia. Incidence rates were calculated using population data from the Florida Office of Vital Statistics. Estimates of raw oyster consumption were obtained from the Florida Behavioral Risk Factor Survey, 1988.

The average annual incidence of raw oyster-associated illness from any *Vibrio* species among raw oyster-consuming adults over 17-years-of-age was estimated to be 10.1/1,000,000 (95% CI: 8.3-11.9). The annual incidence of fatal raw oyster-associated infections from any *Vibrio* species was estimated to be 1.6/1,000,000 oyster-consuming adults (95% CI: 1.3-1.9). In two epidemiological studies, *V. parahaemolyticus* accounted for 77 of 339 reported *Vibrio* infections (Table IV-8) *(57, 83).* Of those 77 persons, 68 reported gastroenteritis and 9 had septicemia. Twenty-nine persons were hospitalized for gastroenteritis with no deaths reported. Eight patients were hospitalized for septicemia and four of those patients died. Patients with septicemia had underlying illness including, but not limited to cancer, liver disease, alcoholism and diabetes *mellitus (57, 83).*

Table IV-8. Clinical syndromes of raw oyster-associated *Vibrio* infections in Florida, 1981-1994

***Vibrio* Species**	**Total Cases**	**Gastroenteritis**	**Septicemia**
V. vulnificus	95	13	82
V. parahaemolyticus	77	68	9
V. cholera Non-O1	74	8	66
V. hollisae	38	35	3
V. mimicus	29	29	0
V. fluvialis	19	19	0

Source of data: *(57, 83)*

In another study, Hlady and Klontz *(58)* reported that of patients with infections, 25% had pre-existing liver disease or alcoholism. These included 75% of the septicemia patients, and 4% of the gastroenteritis patients. Of the remaining septicemia patients, 9 reported having a history of at least one of the following: malignancy, renal disease, peptic ulcer disease, gastrointestinal surgery, diabetes, antacid medication and pernicious anemia. Among the gastroenteritis patients, 74% had none of the above preexisting medical conditions or had insufficient information to classify. Thus, while the prevalence of underlying illness was high in the septicemia patients the majority of patients with raw-oyster associated *Vibrio* gastroenteritis had no underlying conditions. Case series data is available through the Gulf Coast *Vibrio* Surveillance system, which is a unique regional surveillance system that began in 1989 *(89).* Four states participate in this program (AL, FL, TX, LA). Investigators in state and county health departments complete standardized *Vibrio* illness investigation forms on all patients from whom *Vibrio* isolates are reported. *Vibrio* reporting comes from individual physicians,

hospitals, or laboratories. Illness investigation forms contain clinical data concerning signs and symptoms, underlying illnesses, use of medications, as well as epidemiological information concerning seafood consumption in the week prior to illness. Information is then forwarded to the CDC.

During the first year of *Vibrio* surveillance in 1989, *V. parahaemolyticus* accounted for 27 of the 85 reported *Vibrio* illness characterized by gastroenteritis or septicemia *(89)*. *V. parahaemolyticus* was the most prevalent of the *Vibrio* species reported. Twelve of the 27 persons with *V. parahaemolyticus* were known to have eaten raw oysters. One person had septicemia while the remaining 26 persons had gastroenteritis. Oyster-associated infections occurred throughout the year with the peak occurrence in October.

Based upon CDC surveillance data on *V. parahaemolyticus* from 1988-1997 in Alabama, Florida, Louisiana and Texas, the six most common underlying medical conditions associated with infection include diabetes, peptic ulcer, heart disease, gastric surgery, liver disease and immunodeficiency *(6)*. For gastroenteritis, 24% of respondents reported one or more of these six conditions compared with 71% of respondents who had sepsis. In 263 gastroenteritis cases: 7% had diabetes, 6% had peptic ulcer disease, 6% had heart disease, 4% had undergone gastric surgery, 3% suffered from alcoholism, 3% suffered from some form of immunodeficiency, 3% had liver disease, 2% had hematological disease, 2% had some form of malignancy, and 1% had renal disease. Out of 20 septicemic cases, 63% had liver disease, 18% had some form of immunodeficiency, 18% had peptic ulcer disease, 17% had diabetes, 14% suffered from alcoholism, 13% had hematological disease, 12% had undergone gastric surgery, 12% had heart disease, 12% had renal disease, and 11% had some form of malignancy. Among 88 patients with sporadic *V. parahaemolyticus* infection and known food histories, 77 (88%) reported eating raw oysters in the week before illness *(34)*. Of 11 patients with septicemia and known food history, 10 (91%) had eaten raw oysters. Data from the CDC Gulf Coast Surveillance System from 1997 to 1998, were limited only to those cases that are both culture confirmed and ingestion confirmed and resulted in a subset totaling 107 cases. Of these 107 cases, 5 (5%) involved septicemia in which all five were hospitalized with one death. This is believed by CDC to be a fairly accurate estimation of the overall incidence of septicemia among culture-confirmed *V. parahaemolyticus* infections *(6)*. The presence or absence of underlying conditions was reported by 4 of the 5 septicemic patients; 3 (75%) of whom had underlying conditions. Among the 102 cases with gastroenteritis alone, 27 of 90 (30%) respondents reported being hospitalized for the illness. Patient outcome was reported for 83 patients; one of whom died. The presence or absence of underlying conditions was reported by 79 persons; 29% of these reported underlying conditions. The underlying conditions included liver disease, alcoholism, diabetes, malignancy, renal disease, immunodeficiency, hematological disease, gastric surgery and heart disease.

Based upon active FoodNet data surveillance, CDC estimates that the total number of foodborne *V. parahaemolyticus* cases in the United States for 1996, 1997, and 1998 were 2,683; 9,807, and 5568, (rounded to 2,700; 9,800; and 5,600, respectively) *(129)*. These estimates were derived from the numbers of *Vibrio* cases reported to FoodNet. For the calculations, the reports of *Vibrio* cases with unknown species were included for the estimate of the total number of *Vibrio* cases but was not included in the estimate of the percentage of all *Vibrio* spp. that were *V.*

parahaemolyticus. This assumes that the isolates of unknown species are distributed the same as the isolates of known species. The percentage of *V. parahaemolyticus* cases attributed to being foodborne was estimated at 65%. The 1997 estimates are higher as a result of the increased reporting of cases during the Pacific Northwest outbreak. The variation in estimated cases from year to year is expected since the numbers obtained from FoodNet are very small. During the 1972 shrimp-associated *V. parahaemolyticus* outbreak, a survey revealed that of 72 persons with diarrhea only one sought medical attention *(13)*. Due to underdiagnosing and underreporting of cases of *V. parahaemolyticus*, the CDC estimates that the total number of cases is equal to 20 times the reported cases *(94)*. In a CDC random survey of Gulf Coast clinical laboratories, only 20% of the laboratories routinely used selective agar for isolating *Vibrio* species *(34)*.

Geographic distribution

As mentioned earlier, *V. parahaemolyticus* was first identified as a foodborne pathogen in Japan in the 1950s *(48)*. By the late 1960s and early 1970s, *V. parahaemolyticus* was recognized as a cause of diarrheal disease worldwide. Prior to 1994, the incidence of *V. parahaemolyticus* infections in Japan had been declining, however, from 1994 to 1995 there were a total of 1,280 reports of infection due to *V. parahaemolyticus (9)*. During this time period, the incidents of *V. parahaemolyticus* food poisoning outnumbered those of *Salmonella* food poisoning. For both years, the majority of the cases occurred in the summer, with the largest number appearing in August. Food poisoning due to *V. parahaemolyticus* in Japan is usually restricted to relatively small-scale outbreaks involving fewer than 10 cases. From 1996-1998, there were 496 outbreaks, 1,710 incidents and 24, 373 cases of *V. parahaemolyticus* reported. The number of cases of *V. parahaemolyticus* food poisoning cases doubled in 1998 as compared to 1997 and again exceeded the number of *Salmonella* cases *(9)*. Similar to the 1994-1995 period, outbreaks were more prevalent in the summer with a peak in August with few outbreaks during winter months. Boiled crabs caused one large-scale outbreak, involving 691 cases. The majority of outbreaks were small in scale but occurred frequently. The increased incidence during 1997-1998 has been attributed to an increased incidence of serovar O3:K6.

A hospital-based active surveillance study of *V. parahaemolyticus* infections in Calcutta, India, was conducted from 1994-1996, and identified 146 patients *(109)*. The incidence suddenly increased in February of 1996 and remained elevated until August of that year when surveillance ended. The increased incidence of *V. parahaemolyticus* infections was associated with an increased prevalence of O3:K6 strains. This serovar had not been isolated in Calcutta prior to February of 1996. The incidence of diarrhea due to *V. parahaemolyticus* strain O3:K6 accounted for 63% of the strains isolated from patients in Calcutta between September 1996 and April 1997. The virulence of the O3:K6 strains isolated from travelers arriving in Japan from Southeast Asian countries was indistinguishable from O3:K6 strains found in Calcutta, India *(92)*.

Implicated Foods

Vibrio organisms concentrate in the gut of filter-feeding molluscan shellfish such as oysters, clams, and mussels where they multiply and cohere. Although thorough cooking destroys these

organisms, oysters are often eaten raw and are the most common food associated with *Vibrio* infection in the United States *(57)*. However, there have been reports of *V. parahaemolyticus* infections associated with other seafood, including crayfish, lobster, shrimp, and crab. One such report was a case-controlled study of sporadic *Vibrio* infections in two coastal areas of Louisiana and Texas conducted from 1992-1993, in which crayfish consumption was reported by 5 of 10 persons affected with *V. parahaemolyticus* infection *(16)*. Outbreaks of *V. parahaemolyticus* gastroenteritis aboard two Caribbean cruise ships were reported in 1974 and 1975 *(87)*. The outbreaks were most likely caused by contamination of cooked seafood by seawater from the ships' seawater fire systems. In 1972, an estimated 600 of 1,200 persons who attended a shrimp feast in Louisiana became ill with *V. parahaemolyticus* gastroenteritis *(13)*. Samples of uncooked shrimp tested positive for the organism. Three outbreaks occurred in Maryland in 1971 *(32)*. Steamed crabs were implicated in two of the outbreaks after cross-contamination with live crabs. The third outbreak was associated with crabmeat that had become contaminated before and during canning. Recently, sampling studies in the Adriatic Sea demonstrated the presence of *V. parahaemolyticus* in fish, mussels and clams *(10)*.

Consumption

The purpose of this segment is to delineate the factors concerning the consumption of raw molluscan shellfish containing *V. parahaemolyticus.*

Frequency of Consumption and Amount of Raw Molluscan Shellfish Consumed

Intake data for molluscan shellfish are readily available from a number of governmental and non-governmental sources. However, because raw shellfish is not a commonly consumed food (~10- 20% of the population will consume shellfish raw at least once during a year), the data are typically based on very few eaters reporting consumption. The USDA Continuing Survey of Food Intake by Individuals (CFSII) *(135)* and the food frequency survey conducted by the Market Research Corporation of America (MRCA) *(36)* suggest that raw oysters are consumed on average approximately once every 6 weeks. The mean amount of raw oysters consumed at a single serving is 110 grams, approximately one-half dozen raw large Eastern oysters *(128)*. The distribution of shellfish intake will be derived from food intake surveys, food frequency surveys, and from reported landings of shellfish and industry estimates of the percentage of shellfish consumed raw**.**

Population at Risk

Anyone who consumes shellfish raw is "at risk" for infection by *V. parahaemolyticus*. An FDA telephone survey completed in 1993 and repeated in 1998 has shown that consumption of raw shellfish is not uniformly distributed *(90)*. A higher percentage of men consume raw oysters than women (16% vs. 7%), and raw shellfish consumption is higher for those living along the coastline of the United States than for those living inland (22% vs. 13%). The trends in raw shellfish consumption, as evidenced in the 1998 FDA survey is toward lowered consumption of raw shellfish. This may be the result of education efforts by the Agency concerning the risks associated with the consumption of raw or undercooked protein foods, such as beef, chicken, eggs, and shellfish. Paradoxically, raw shellfish consumption is highest among those with the

highest education levels, and the trend toward reduction in raw shellfish consumption over the last 5 years is smallest in this education group.

Oyster Landings Data

The time of year of consumption was considered in the risk assessment, as most infections occur during warm months, that is, a person consuming the raw oysters in July is at higher risk than the same person consuming the same amount in December. The location of harvest is also important, with most landings of oysters occurring in the Gulf, particularly off the coast of Louisiana.

V. HAZARD CHARACTERIZATION/DOSE-RESPONSE

Hazard characterization describes the adverse effects on the host of a particular substance, organism, or other entity. It may be a quantitative and/or qualitative evaluation of the nature of these adverse effects. Dose-response, which is quantitative, is the relationship of the levels of *V. parahaemolyticus* ingested with the frequency and magnitude of illness. Human dose-response relationships for *V. parahaemolyticus* can be derived directly from human clinical feeding trials and epidemiological (outbreak) investigations, if sufficient data exist. To date, little information in terms of estimated exposure doses has been obtained from outbreak investigations. For *V. parahaemolyticus*, dose-response data is available directly from several human clinical feeding trials. However, these studies were performed prior to 1974 with uncharacterized strains, antacid administration and with no information on the immune status of the volunteers in terms of preexposure to *V. parahaemolyticus*. Even partial immunity to *V. parahaemolyticus* could raise the observable infectious dose compared to what may occur in the general population. In addition, in outbreak settings, lower doses of *V. parahaemolyticus* may cause illness if the organism is mixed with food that can buffer the gastric acidity thereby lowering the infectious dose. It is unlikely that any additional human feeding studies with *V. parahaemolyticus* will be undertaken due to the observed cardiotoxicity of TDH in animal models *(60, 119)*. In the absence of additional data for *V. parahaemolyticus*, an alternative for dose-response modeling is to select an appropriate surrogate bacteria for which additional dose-response data is either available or can be generated. Additional information considered essential would include greater low dose exposure data (including biomarkers) and the role of the food matrix on dose-response relationships. Information on non-O1 *V. cholerae* is provided as a possible surrogate organism.

Since limited human clinical information was available, it was assumed that all *V. parahaemolyticus* clinical isolates are equally virulent *(105)* and that the primary virulence factor is TDH *(105)*. This may be modified as new data become available that identify new virulence determinants. In particular, recent data from British Columbia *(77)* may suggest an association of urease positive strains with clinical isolates, which may or may not be TDH positive.

Animal models using *V. parahaemolyticus* or a surrogate organism can be used as surrogates to provide a basis for extrapolating dose-response estimates for humans. Animal models can also be used to assess the virulence potential of different strains and serotypes, susceptibility of the sensitive subpopulation (i.e., immune compromised), and to study the role of specific virulence determinants. Several *V. parahaemolyticus* animal models have shown the virulence potential of TDH negative strains *(59,84)*. However, it remains to be determined whether the virulence potential indicated for TDH^- strains also applies to humans. The effect of food matrices and other environmental factors on virulence and the dose-response relationship can be evaluated more readily in animal models.

Human Clinical Feeding Studies

Feeding trials with *V. parahaemolyticus*

In the study by Takikawa *et al. (125)*, a Kanagawa-positive strain (production of TDH, as observed on a blood agar plate) caused diarrhea in 1 of 2 individuals fed a dose of approximately 10^6 cells. Diarrhea occurred in 2 of 2 individuals fed approximately 10^7 cells *(125)*. Ingested doses were estimated assuming that *V. parahaemolyticus* cultures can reach maximum growth densities of approximately 10^{10} cells per milliliter.

Three Kanagawa negative strains (no production of TDH as observed on a blood agar plate) isolated from cases of gastroenteritis were fed to groups of four volunteers each. No illness was observed in any of the groups at doses as high as 2×10^{10} cells. A Kanagawa positive strain also isolated from a gastroenteritis case produced no symptoms at a low dose of 200 viable cells. Abdominal discomfort occurred in 1 of 4 volunteers at a dose of 2×10^5 viable cells, and 2 of 4 volunteers experienced abdominal discomfort and diarrhea at 3×10^7 viable cells. All volunteers received antacid tablets prior to challenge with cultures suspended in gelatin *(116)*.

Feeding tests carried out with 15 Kanagawa negative strains isolated from fish produced no illnesses when doses as high as 10^9 viable cells were used *(115)*.

Although never published, a personal communication is cited *(71)* that reports it took 6 to 8 hours incubation for a *V. parahaemolyticus* hemolytic variant to cause disease while an non hemolytic variant required approximately 18 hours to cause disease after challenge. The infecting dose was stated to be approximately 10^6 organisms. No information is provided about the strain and it is unknown if the strain was truly a TDH^- strain since no genetic analysis of the strain was performed.

Feeding trials with non-O1 V. cholerae

One of three strains of non-O1 *V. cholerae* fed to healthy volunteers caused no diarrhea in 2 volunteers fed 10^5 cells, 2 of 3 fed 10^6, 1 of 2 fed 10^7 and 3 of 3 fed 10^9. Two other strains produced no disease at doses as high as 10^9 cells *(98)*.

V. cholerae O139 Bengal fed to volunteers caused diarrhea in 2 of 4 fed 10^4 cells and in 7 of 9 fed 10^6 cells *(97)*.

Animal Models

For possible inclusion in future modeling, animal dose-response data and several factors influencing the infectious dose of *V. parahaemolyticus*, using animal studies are described in this section.

Animal models for *V. parahaemolyticus*

Suckling rabbits infected orally with a Kanagawa-positive strain at doses of 10^9 to 10^{10} had positive blood cultures in 9 of 36 tested, positive spleen cultures in 11 of 21 tested and positive liver cultures in 14 of 21 tested *(20)*. Similar doses of a Kanagawa negative crab isolate were negative for bacteremia, liver or spleen invasion in all 12 animals challenged *(20)*.

Combined results of seven experiments in which mice were challenged intraperitoneally with 1 of 7 strains (four TDH^+ strains and three TDH^- negative strains) resulted in 0 deaths with a dose of 10^5 cells; 4% deaths with a dose of 10^6; 61% deaths with a dose of 10^7, and 90% deaths with a dose of 10^8 cells *(59).*

Combined results of two experiments in which mice were challenged orally with 1 of 2 TDH^+ strains resulted in 38% deaths with a dose of 10^7 cells, 57% deaths with a dose of 10^8 and 80% deaths with a dose of 10^9 cells *(59)*. There were no significant differences in mortality between the TDH^+ and TDH^- strains at any of the doses.

In rabbit ileal loop studies the effective dose required to produce ileal loop dilation in 50% of rabbits for three Kanagawa positive strains ranged from 2.6×10^5 to 7.7×10^6 cells. It was estimated that the initiation of positive loops occurred with doses from 10^2 to 10^5 cells *(134).*

Animal models for other *Vibrio* spp.

Severity of disease increased and time until death decreased in rabbits when a non-O1 *V. cholerae* strain shown to cause diarrhea in volunteers, was administered in increasing doses of 10^3, 10^4 and 10^9 cells using the removable intestinal tie adult rabbit diarrhea (RITARD) model *(114)*.

Fluid accumulation, diarrhea, and mortality of strains of non-O1 and O1 *V. cholerae* and *V. fluvialis* was studied in orogastrically challenged suckling mice. The 50% lethal dose values ranged from 10^7 to 10^9 CFU. The effective oral dose producing stained feces was about 1 log lower than the LD_{50} dose for each strain *(106)*.

Factors Influencing the Infectious Dose of *V. parahaemolyticus*

- Bacterial/Virulence Factors
  - The percentage of positive blood cultures in orally challenged suckling rabbits increases when strains that had been previously passed through suckling rabbits are used *(20).*

- Host Factors
  - Production of the TDH is enhanced by the presence of bile acids *(112).*
  - Iron-limiting conditions enhance the virulence of *V. parahaemolyticus* in mice after intraperitoneal challenge *(33).*
  - Acid adaptation enhances the virulence of *V. parahaemolyticus* in mice after oral challenge *(144)*.

- Food/Environmental Factors
  - Studies with *V. cholerae* O1 indicate that a food matrix, such as cooked rice, provides buffering capacity and may have substantive impact on dose-response relationships *(88)*.
  - Addition of 5% mucin to the inoculum enhances the virulence of *V. parahaemolyticus* in mice following intraperitoneal challenge *(59)*.

Modeling of the Public Health Module

The Public Health Module predicts distributions of illness based on the distribution of serving size (oysters per serving and weight of oysters), the predicted levels of pathogenic *V. parahaemolyticus* at time of consumption and an estimated relationship between the probability of illness and ingested number of organisms per serving (Fig. V-1). For the purpose of modeling, only Kanagawa-positive *V. parahaemolyticus* strains have been considered as being pathogenic. In most studies, greater that 90% of the *V. parahaemolyticus* strains isolated from the stools of symptomatic cases are found to be Kanagawa-positive *(34, 145)*. In contrast, less than 1% of strains isolated from the environment are found to be Kanagawa-positive. To date no other virulence determinant has been correlated with clinical disease. The predicted density of pathogenic *V. parahaemolyticus* at time of consumption is determined in the Post Harvest Module under assumptions of current industry practice and possible mitigations. Distributions of number of organisms ingested are obtained by multiplying the estimated densities of pathogenic *V. parahaemolyticus* by serving size.

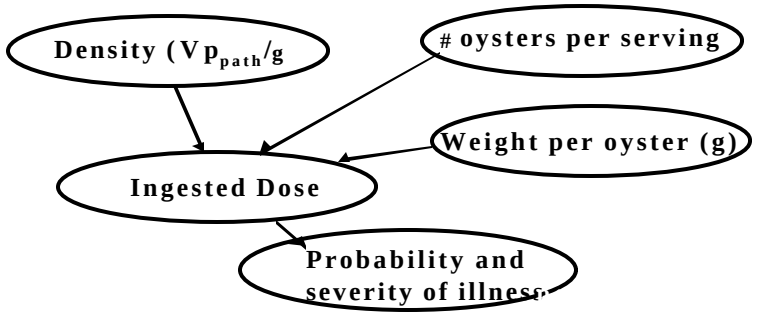

Figure V-1. Schematic depiction of the Public Health Module of the *V. parahaemolyticus* (Vp) risk assessment model.

Human clinical trials with Kanagawa-positive *V. parahaemolyticus* strains conducted prior to 1974 were evaluated for the purpose of determining a dose-response relation for converting distributions of ingested doses into distributions of risk per serving *(2, 116, 125)*. However, in consideration of oyster consumption statistics and predicted levels of pathogenic *V. parahaemolyticus* at time of consumption, the dose-response (e.g. ID_{50}) in the human feeding trials was found to overpredict the CDC estimates of annual number of illnesses by a factor of 10. This strongly suggests that the dose-response under conditions of normal population exposure is different than under the conditions of the feeding trials. Possible reasons for the difference include food matrix or immunological effects of preexposure to the organism including antibodies/vaccines to the organism *(88)*. Consequently, a plausible dose-response was obtained by shifting the dose-response by a factor of 10, estimated from the feeding trials so as to be generally consistent with CDC estimates of illness. The predicted number of illnesses associated with oysters from each region and season were derived based on the projected number of raw oyster-servings. The probable number of illnesses associated with the oysters landed from each region was determined rather than the number of illness within each geographic region. Obviously, the number of illnesses occurring within a given region and season is due to oysters originating from various regions of the country.

An estimate of the distribution of risk per serving due to oysters originating from various locations would be obtained as a weighted average of the distributions of risk associated with oysters harvested from each region. However, a reliable estimate of the extent of interregional transport, which would be necessary to estimate the appropriate weights, was not identified.

Distribution of dose of pathogenic *V. parahaemolyticus* per serving

The distribution of the dose of pathogenic *V. parahaemolyticus* ingested per serving was estimated based on distributions of (a) the number of oysters consumed; (b) the weight of oysters consumed; and (c) the density of pathogenic *V. parahaemolyticus* per g output from the Post Harvest Module. The distribution of densities of pathogenic *V. parahaemolyticus* obtained in the Post Harvest Module is projected to represent the variation of average density over collections of oysters being consumed on any given occasion. This is appropriate in so far as total *V. parahaemolyticus* densities measured in the DePaola *et al. (38)* study were average densities over composites of twelve oysters and this is a typical serving size per serving. Implicitly, a collection of oysters being consumed on any given occasion is assumed to have originated from the same region.

The distribution of the number of oysters consumed per serving is shown in Figure V-2. The most typical serving sizes were 6, 12 and 24 oysters. This frequency distribution is based on a 1994 Florida consumer survey conducted by the Florida Agricultural Market Research Center (University of Florida) *(36)*. To obtain the estimate of the distribution of meat weight per serving, estimated distributions of meat weight for servings of a given size were combined with the distribution of serving size.

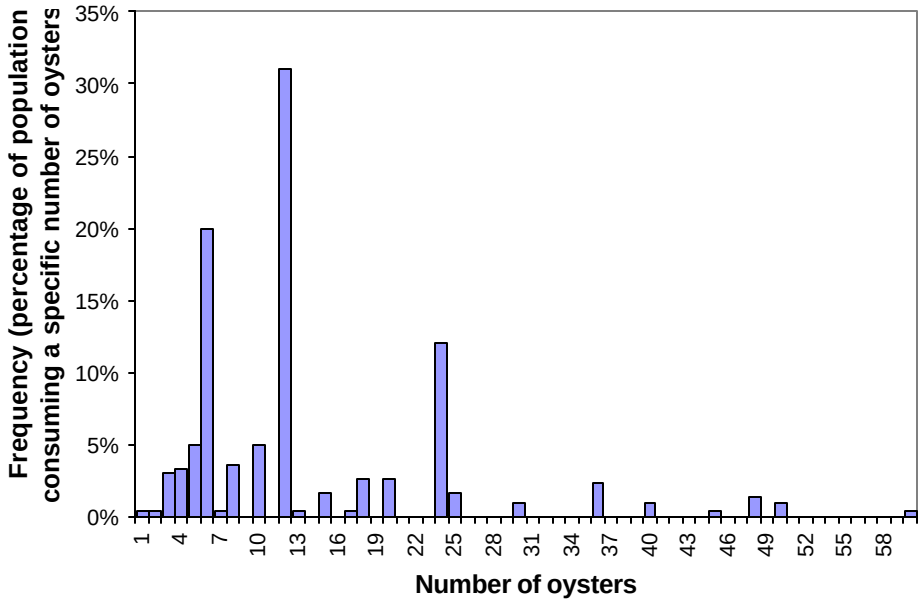

Figure V-2. Observed frequency of number of oysters consumed per serving (University of Florida consumption survey) *(36)*.

Based on review of available data, the distribution of the meat weight corresponding to a serving of n oysters was adequately approximated as a normal distribution with mean and variance of $n*\mu$ and $n*\sigma^2$, respectively, where μ and σ^2 are the mean and variance of meat weight per single oyster. The distribution was truncated to eliminate values below 15*n and above 35*n grams on the assumption that it was unlikely that individual oysters would have meat weight outside of the range of 15 to 35 grams. An estimate of the mean and standard deviation of meat weight of individual Gulf oysters is 26 and 7.3 grams respectively *(37)*. When combined with the distribution of serving size, the resulting distribution of meat weight per serving was considered typical of all regions.

Although, oysters harvested in the Pacific Northwest are somewhat larger than Gulf oysters the meat weight per serving is unlikely to vary substantially across different regions of the country. The distribution of number of oysters per serving is based on a survey of Florida consumers who would have been consuming predominately Gulf oysters. Therefore it is appropriate to combine this distribution with the distribution of meat weight of Gulf oysters.

The distribution of the number of pathogenic *V. parahaemolyticus* ingested per serving was determined by multiplying the distribution of the densities of pathogenic *V. parahaemolyticus* per gram oyster meat (the average density projected for composites of oysters) and the distribution of meat weight per serving, as determined above.

Number of raw oyster servings

The number of raw oyster servings associated with oysters harvested from different regions and seasons was estimated based on National Marine Fisheries Service (NMFS) landings data. The total monthly oyster landings reported by NMFS were averaged over the period 1990 to 1998, then grouped by season and region (Table V-1). Oyster landings are reported by NMFS as pounds of oyster meat weight. Industry figures suggest that 50% of the harvest is consumed raw with little variation across different seasons. Therefore, the projected number of illnesses which may occur have been based on the assumption that 50% of the harvest is consumed raw. The actual fraction of the landings consumed raw in any particular year may vary somewhat with a range of 40% to 60% being reasonable. However, total landings also vary from year-to-year in a manner that is not predictable due to the influence of other factors (e.g. closures due to water quality, effect of parasites). Oyster landings have been generally increasing over the past 5 years but it is uncertain to what extent this trend will continue in the future.

Table V-1. National Marine Fisheries Service (NMFS) average yearly oyster landings 1990-1998

Location	Average Number of Pounds of Oyster Meats Harvested				
	Winter (Jan - March)	**Spring** (April - June)	**Summer** (July - Sept)	**Fall** (Oct - Dec)	**Total**
Atlantic Northeast	2,112,000	714,000	676,000	3,710,000	7,212,000
Mid-Atlantic	946,000	125,000	66,000	1,492,000	2,629,000
Gulf Coast					
Louisiana	2,751,000	2,630,000	2,854,000	2,769,000	11,004,000
Other States	2,096,000	1,393,000	847,000	2,358,000	6,694,000
Gulf Total	4,848,000	4,023,000	3,701,000	5,127,000	17,699,000
Pacific Northwest	2,402,000	1,682,000	1,379,000	3,181,000	8,644,000
Total	10,308,000	6,544,000	5,822,000	13,509,000	36,183,000

Source of data: http://www.nmfs.noaa.gov/

The number of raw servings associated with oysters from each harvest region and season were estimated as:

$$\frac{L_i * f}{W * S}$$

where L_i are the regional and seasonal total landings expressed in units of meat weight (grams), f is the percentage of oysters consumed raw, W is the average meat weight per oyster (grams), and S is the average number of oysters per serving (14.7 based the 1994 FL consumer survey) *(36)*.

Dose-Response

Generally, the most appropriate data upon which to estimate the dose-response relationship of a bacterial pathogen would be the outcome of feeding trials with human subjects. Although subjects selected for feeding trials tend to be healthier than the general population, the magnitude of the uncertainty when extrapolating from such data is generally less than that associated with the extrapolation of dose-response determined from animal studies. In particular, animal data were not utilized in our initial modeling efforts because the endpoint was death rather than illness. Measures of the severity of illness used in animal studies often do not correspond with definitions of human illness on which reporting statistics are based.

For pathogenic *V. parahaemolyticus*, as identified by the Kanagawa test, several human clinical feeding trials were conducted prior to 1974. Epidemiological investigations of *V. parahaemolyticus* provide additional information on plausible dose-response of gastroenteritis but are somewhat limited due to the lack of data concerning ingested doses of pathogenic strains associated with reported cases of illness (e.g. epidemiological traceback studies). Epidemiological case series data do provide valuable information which can be used to estimate the likelihood of illness progressing to more severe outcomes (i.e., septicemia, death) for both immune compromised and otherwise healthy populations. Estimates pertaining to the severity of illness based on epidemiological data are presented in the next section.

The dose-response relationship for illness (gastroenteritis or septicemia) was estimated based on the 1974 study of Sanyal and Sen *(116)* augmented by data from studies by Takikawa *(125)* and Aiso *(2)*. Overall, 5 of the 16 subjects who received higher ingested doses of Kanagawa positive strains developed symptoms of gastroenteritis in these studies. No severe outcomes were observed. Dose-response, using all the doses from the three studies, was characterized by fitting several dose-response models chosen to span the range of extremes of model extrapolated risks at projected levels of population exposure.

The selected models were the Beta-Poisson, Gompertz, and Probit (Log-normal). The Gompertz and the Probit are generalized linear models. For these two models, the linear predictor was chosen to be a linear function of $\log_{10}$ ingested dose. The mathematical form of these dose-response models is shown in Table V-2.

Table V-2. Dose-response models of the relationship between probability of illness and number of *V. parahaemolyticus* organisms ingested.

Dose-response model	Risk of illness as a function of dose [a]
Beta-Poisson	$\Pr(ill \mid d) = 1-(1+\frac{d}{\beta})^{-\alpha}$
Probit	$\Pr(ill \mid d) = \Phi(\alpha + \beta * \log_{10}(d))$
Gompertz	$\Pr(ill \mid d) = 1-\exp[-\exp[\alpha + \beta * \log_{10}(d)]]$

[a] α and β are the location and shape (steepness) parameters, respectively, for the Probit and Gompertz models; α and β are the shape (steepness) and location parameters, respectively, for the Beta-Poisson; Φ denotes the cumulative distribution function of a standard normal random variable

The maximum likelihood estimates (MLE) of the Beta-Poisson, Gompertz and Probit dose-response models are shown in Figure V-3. For example, at a dose of 100 (2 $\log_{10}$) *V. parahaemolyticus* organisms, the Beta-Poisson model predicts a risk of approximately 7 cases of illness per 10,000 challenges at that dose. Best estimates of the risk of illness at ingested doses of less than 10^3 organisms vary by more than 10-fold across this set of plausible models. However, based on the estimates of exposure developed in the Harvest and Post Harvest Modules, the mean (average) exposure to pathogenic *V. parahaemolyticus* exceeds 100,000 cells per serving for the Gulf Coast summer harvest. At this level of exposure the differences between the dose-response models is not substantial. The average dose associated with the Gulf Coast summer harvest is less than 2 $\log_{10}$ below that of ID_{50} estimates for the human feeding trial studies based on any of the three models considered.

Consideration of the predicted density of pathogenic *V. parahaemolyticus,* the number of raw oyster servings for the Gulf Coast summer harvest and the likely number of illnesses occurring (CDC personal communication) *(80),* strongly suggests that the predicted risks per serving based on dose-response curves shown in Figure V-3 are not plausible. Consequently, direct extrapolation of the dose-response under conditions of exposure in the feeding trials is not supported by the epidemiological data. The human feeding trials were conducted under conditions of concurrent antacid administration. For *V. cholerae*, the ID_{50} observed in feeding trials is known to be substantially lower when *V. cholerae* is ingested with antacid versus no antacid *(88)*. The same effect is likely to be the case with *V. parahaemolyticus*. It is also possible that food matrix or immunological effects of preexposure to the organism, including antibodies/vaccines, contribute to the apparent difference in dose-response obtained under experimental versus natural conditions *(88)*. Antibodies to *V. cholerae* increased the ID_{50} *(88)*. Likewise, prior consumption of oysters containing non pathogenic *V. parahaemolyticus,* may also contribute to a higher ID_{50}. It has also been reported, for example, that in rats, high milk fat intake results in higher concentrations of gastric bactericidal lipids, which protect against *Listeria* infection *(123)*. Furthermore, gastrin, the most potent stimulant of gastric acid secretion is released after eating a protein-rich meal, like oysters *(8)*. The increased production

of gastric acid would provide greater protection against infection, thus increasing the infectious dose.

The relevant epidemiological data is summarized by estimates of annual incidence of *Vibrio* illness developed by Mead *et al. (94)* as part of a comprehensive evaluation of the national burden of infectious food-related illness in the United States. Mead *et al. (94)* have estimated an average annual burden of 7,880 *Vibrio* illnesses excluding those due to *V. vulnificus*, and that 65% percent of this total incidence is estimated to be food-related. This estimate was based on frequency of reported cases obtained by passive surveillance from 1988 through 1996 and frequency of reported cases through FoodNet in 1996 as extrapolated to the 1997 population. This total illness caused by non-*vulnificus Vibrio* spp. is based on an estimate of 20 to 1 underreporting and underdiagnosing of illness *(80, 94)*.

The reported cases of illness attributable to *V. parahaemolyticus* in recent years is a component of the data on which total incidence of non-*vulnificus Vibrio* illness is based on the information reported by Mead *et al. (94).* Specific yearly estimates of total illness attributed to *V. parahaemolyticus* for 1996, 1997 and 1998 are 4128, 15088 and 8567, respectively *(94)*, based upon active FoodNet data surveillance. Assuming that 65% of these illnesses are food-related, CDC estimates that the total number of foodborne *V. parahaemolyticus* cases in the United States for 1996, 1997 and 1998 was approximately 2700, 9800, and 5600, respectively.

Given these estimates of annual illness rate it was determined that at least a 10-fold increase of the ID_{50} estimated with respect to the feeding trials was necessary to infer a dose-response consistent with the epidemiology. It is possible that the true ID_{50} for the general population is even greater than implied by this adjustment but this uncertainty was not evaluated in the present risk assessment.

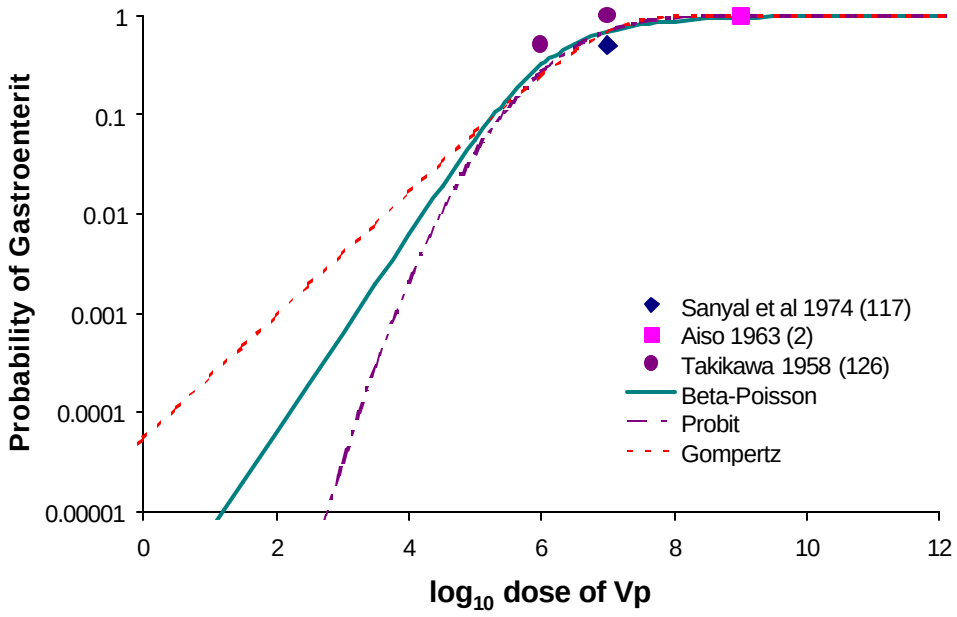

Figure V-3. Maximum likelihood estimates (MLE) of the Beta-Poisson, Gompertz, and Probit dose-response curves based on pooled data from human feeding studies of *V. parahaemolyticus* (Vp).

The uncertainty with regard to which model is most appropriate for risk extrapolation is often referred to as a structural uncertainty. An additional source of uncertainty in dose-response is referred to as parameter or statistical uncertainty. This uncertainty derives from the estimation of the parameters of a given model based on a small sample of observations. Parameter uncertainty is present even if there is no structural uncertainty.

Parameter uncertainty in the extrapolated risk for the Beta-Poisson, Gompertz and Probit models was evaluated by nonparametric bootstrapping (i.e., replication) of feeding trial outcomes. Using this procedure, for each possible bootstrap, the models were refit to obtain a distribution of parameter estimates corresponding to the possible (unrealized) outcomes of the human feeding trials. The series of parameter estimates obtained are weighted by the probability of the corresponding bootstrap outcomes. For each model, the distribution of parameter estimates obtained defines the uncertainty of predictions or extrapolations conditional on the structure of the model (i.e., in the absence of structural uncertainty). For example, the parameter uncertainty associated with the model predicted infectious dose level corresponding to a risk of 10^{-3} is shown in Figure V-4 (as estimated by 1,000 bootstrap samples) for each of the three models considered. As can be seen, the uncertainty distribution of this particular benchmark dose has somewhat the same spread (or range) for both the Gompertz and the Probit. However, since the distribution for the Gompertz places much more weight on the extremes of the range, the uncertainty of the estimate is much greater for the Gompertz than for the Probit

model. The uncertainty is somewhat less for the Beta-Poisson. The means of all three of these distributions are comparable to the predictions of the MLEs for these models, as shown in Figure V-4.

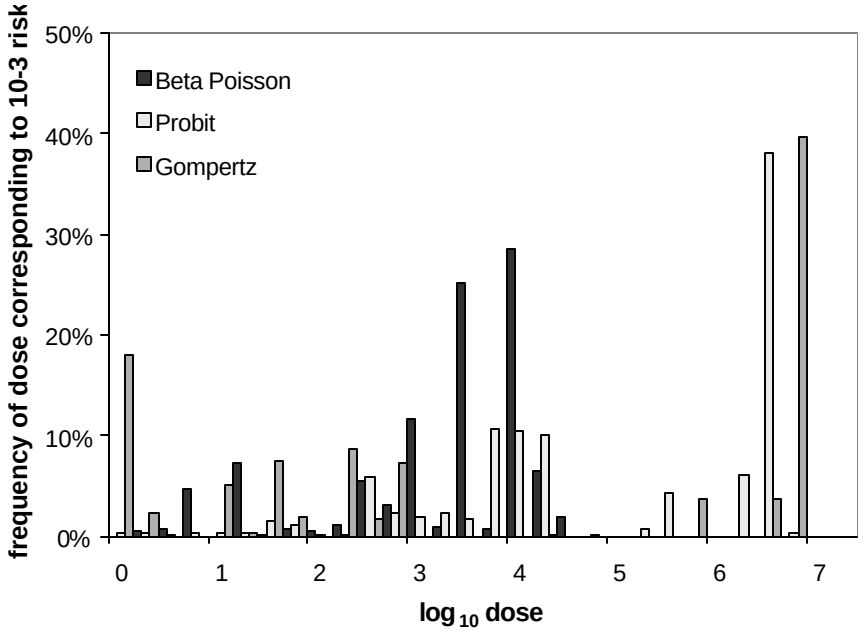

Figure V-4. Uncertainty distribution of infectious dose of *V. parahaemolyticus* corresponding to 10^{-3} risk for Beta-Poisson, Gompertz, and Probit dose-response models.

Parameter uncertainty was evaluated in the risk assessment by simulating the uncertainty of the estimate of risk associated with any dose consumed. For each of the models considered, the risk of illness at a specific dose was considered to have a distribution determined by the bootstrap distribution of parameter estimates. Given a risk of illness on an eating occasion/serving, whether or not illness occurs was then modeled as a Bernoulli random trial with the corresponding risk of illness as parameter. Structural uncertainty was evaluated by conducting multiple simulations using the different structural forms for the dose-response extrapolation.

Severity of Illness

The output of the Public Health model is the probability distribution for the number of illnesses expected to occur from consumption of oysters from different sources (seasons/regions). These predictions assume that the probability of infection at any given ingested dose is the same for all consumers. Clearly, most infections go unreported. In the Gulf Coast states where *V. parahaemolyticus* infections are more actively identified, individuals who are immune compromised or have liver disease are notably over represented in the case series of culture-confirmed illness. There are two possible explanations for this observation. It is possible that

there exists a sensitive subpopulation that is more susceptible to infection at any given dose. However, it is more likely that there exists a sensitive subpopulation which, given the occurrence of infection, is more likely to progress to severe outcomes requiring the attendance of a physician. That is to say, the overrepresentation of immunocompromised individuals in culture-confirmed case series is likely to be a reporting phenomenon driven by the severity of illness.

For the purpose of the risk assessment, we have assumed that there is no sensitive subpopulation with respect to the occurrence of an infection leading to gastroenteritis or septicemia. However, given the occurrence of illness, we assume that it is more likely that the infection leads to severe outcome (e.g. septicemia or death) among individuals with an underlying condition. The best available information to quantify this differential likelihood of severe outcome is the CDC database of culture-confirmed *V. parahaemolyticus* cases in the Gulf Coast states.

Estimates of the conditional probabilities of septicemia and death following infection leading to illness in healthy and immune-compromised individuals can be estimated by using Bayes theorem*(46)* and the frequency of underlying conditions among identified culture-confirmed *V. parahaemolyticus* cases. Specifically, the calculation uses Bayes theorem in the form:

$$\Pr(outcome|condition) = \frac{\Pr(condition|outcome) * \Pr(outcome)}{\Pr(condtion)}$$

where, for example, Pr(outcome|condition) denotes the probability or frequency of an outcome among a population of individuals grouped by health status (condition). All factors on the right hand side of the equation are identifiable from the epidemiological data.

The frequency of underlying conditions was identified among 107 oyster-related culture-confirmed *V. parahaemolyticus* cases (sporadic- and outbreak-related) occurring during 1997 and 1998 in the Gulf Coast States *(6)*. The statistics of the case series were:

- 5 septicemia
- 1 death

Of cases with available information:

- 23 of 79 (29%) cases occurred in individuals with underlying chronic conditions
- 27 of 90 (30%) gastroenteritis cases were hospitalized
- 3 of 4 (75%) septicemia cases had an underlying chronic condition

Substituting the appropriate observed frequencies into the above equation provides estimates of the probabilities of progression to more severe outcomes conditional on culture-confirmed illness. For example, the probability of septicemia occurring following culture-confirmed illness among individuals with underlying chronic conditions is estimated as follows:

$$\Pr(septicemia \mid sensitive) = \frac{\Pr(sensitive \mid septicemia) * \Pr(septicema)}{\Pr(sensitive)}$$

$$= \frac{3/4 * 5/107}{23/79} = 0.12$$

The probability of septicemia occurring after culture-confirmed illness in healthy individuals is estimated in a similar fashion. Overall, the estimated conditional probabilities of severe outcomes based on the CDC data are:

$$\Pr(septicemia \mid sensitive \,\&\, culture\ confirmed) = 0.12$$
$$\Pr(septicemia \mid nonsensitive \,\&\, culture\ confirmed) = 0.0165$$
$$\Pr(septicemia \mid culture\ confirmed) = 0.047$$
$$\Pr(death \mid septicemia) = 0.2$$

These estimated frequencies pertain to the population of culture-confirmed illnesses that may occur in any given year. Certain assumptions are necessary to estimate the frequency of severe outcomes occurring among the population of all *V. parahaemolyticus* illness, regardless as to whether it is culture-confirmed or not.

Clearly, there is a selection bias towards more severe outcomes in the culture-confirmed case series. It is unlikely that a significant fraction of cases of septicemia would go undiagnosed. Overall, considering the less severe outcomes, it is estimated that 1 in 20 cases (5%) of *V. parahaemolyticus* illness are reported or diagnosed in the Gulf Coast states (CDC, personal communication) *(6).* Thus we would estimate approximately 2140 illness occurring over a time period during which 5 septicemia cases were identified. Assuming that all septicemia are culture confirmed, the Bayes calculation for the probability of progression to septicemia among sensitive individuals who have become ill is:

$$\Pr(septicemia \mid sensitive \,\&\, ill) = \frac{3/4 * 5/2140}{23/79} = 0.006$$

If only 50% of septicemia are reported and culture-confirmed, the corresponding estimate is 0.012. For healthy individuals, the estimated rates of septicemia following illness are 0.0008 and 0.0016, assuming complete and 50% underreporting, respectively.

Given estimates of conditional probabilities, the frequency of septicemia can be simulated in the model based on the relative frequency of consumption of raw oysters by sensitive and healthy individuals. Approximately 7% of the general population have an underlying condition predisposing to *V. vulnificus* infection *(82).* The same set of conditions would likely predispose to more severe *V. parahaemolyticus* illness. If sensitive individuals consume raw oysters at the same frequency as the general population then the overall risk of septicemia occurring is the weighted average of the conditional probabilities of septicemia for sensitive and healthy individuals.

$$\Pr(septicemia \mid ill) = 0.07 * \Pr(septicemia \mid ill, sensitive) + 0.93 * \Pr(septecemia \mid ill, healthy)$$

The distribution of the probable number of septicemia which may occur in a given year is therefore a binomial with size parameter equal to total number of illnesses and the probability parameter equal to the overall risk of septicemia following illness. Implicitly, this probability of septicemia (and the probability of other severe outcomes) has been assumed to be independent of the dose leading to infection and illness.

VI. RISK CHARACTERIZATION

Risk characterization is the integration of the Exposure and Dose-Response assessments (Figure VI-1). This phase describes the probability of illness caused by consumption of oysters harboring pathogenic *V. parahaemolyticus*, and discusses the impact of the risk assessment. In this section, the predicted number of illnesses associated with each region and season are presented based on the model assumptions discussed in the previous sections. The distributions of the probable number of illnesses that may occur associated with regional/seasonal harvests, presented here, were derived based on the projected number of occasions when raw oysters were consumed.

Simulation Results

As stated previously, the overall structure of the risk simulation was divided into three modules: Harvest, Post Harvest, and Public Health. The Harvest Module simulated the variation in total and pathogenic *V. parahaemolyticus* densities as a function of underlying environmental conditions. Analysis of the effects of salinity on the levels of pathogenic *V. parahaemolyticus* in the Harvest Module, suggested that salinity was not an important variable. The salinity component was removed and a model developed for *V. parahaemolyticus* growth based solely on water temperature. The output of the Harvest Module was the distribution of total and pathogenic *V. parahaemolyticus* densities in oysters at the time of harvest. The Post Harvest Module simulated the effect of current oyster handling practices and the possible effects of mitigations to derive predictions of the distribution of total and pathogenic *V. parahaemolyticus* densities at time of consumption. The Public Health Module estimated the distribution of the probable number of illness, which may be expected to occur within any given region and season on the basis of the predicted distribution of pathogenic *V. parahaemolyticus* densities at time of consumption. Throughout the simulation we utilized derived distributions of influential parameters and relationships between parameters to obtain estimates of the distribution of pathogenic *V. parahaemolyticus* densities at various stages along the pathway from harvest to consumption. Figure VI-1 shows a schematic representation of all the parameters used in the simulations for each module and how the output of each module becomes a parameter for the following module.

The distribution of ingested dose of pathogenic *V. parahaemolyticus* per serving was calculated by combining the distributions of meat weight per serving and distributions of density from the Post Harvest model. This was accomplished by following the Monte Carlo method of resampling from these input distributions and multiplying the sampled values to generate the distribution of consumed doses. Simulated samples from the distribution of ingested doses were then converted to a corresponding distribution of risk per serving. For each region and season, the number of illnesses occurring in 100,000 servings was then simulated as a sequence of independent, but not identically distributed Bernoulli random variables. Although larger simulation sizes are possible, the need to run multiple simulations (e.g. different regions and seasons) necessitated limiting the size of each simulation to a practical level. Predicted numbers

of illnesses were determined by projecting the number of illnesses per 100,000 to the number of servings estimated based on the NMFS landings statistics. The simulations were repeated 50 times to simulate the year-to-year variation in the number of illnesses which may be expected due to the year-to-year variation in such parameters as the distributions of water temperatures and percentage of total *V. parahaemolyticus* which are pathogenic. The website where the worksheet showing the formulae and parameters used for the model can be found is in Appendix II.

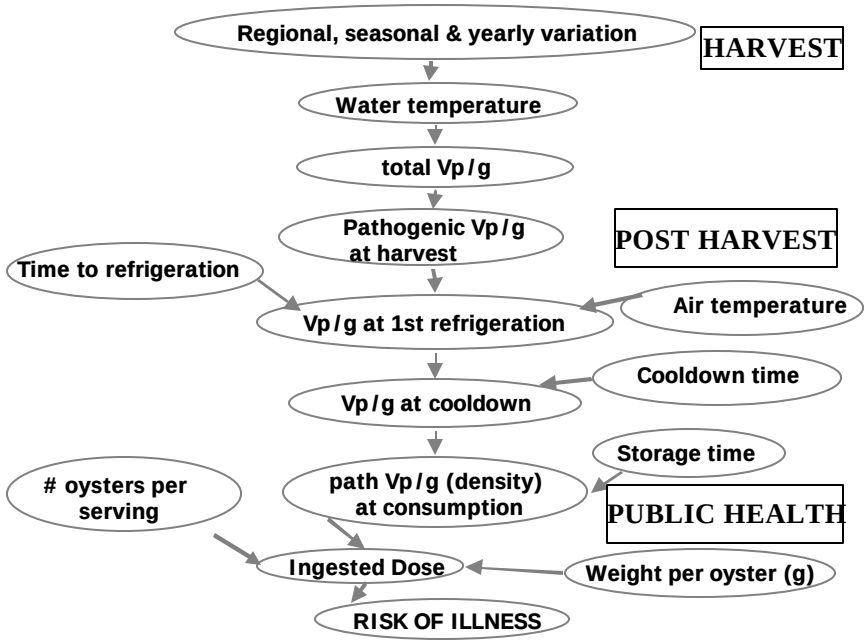

Figure VI-1. Schematic diagram of the *V. parahaemolyticus* (Vp) risk assessment model showing integration of all the modules.

Probable distribution of illness associated with regional/seasonal oyster harvest

The distribution of the probable number of illnesses predicted by the Monte Carlo simulation was somewhat sensitive to the choice of the dose-response model. The difference in model predictions is shown in Figure VI-2 for the Gulf Coast (Louisiana) harvest during the summer. These histograms of the distributions are approximations based on 50 repeated simulations with randomly varying factors such as mean seasonal water temperature and percentage of total *V. parahaemolyticus* which are pathogenic. As shown in Figure VI-2, the predictions of illness under the Gompertz dose-response model are considerably more variable than under either the Beta-Poisson or Probit dose-response models. Preliminary simulations had suggested that a 10-fold shift of the ID_{50} (e.g. in regard to possible food matrix and immunological effects) would be sufficient to make model predictions generally consistent with CDC estimates of annual illness. However, the extent to which the estimate of the ID_{50} for the human feeding trials underestimates the "true" population ID_{50} is not known. If, for example, the difference in ID_{50} in the feeding trials versus conditions of general exposure was 10,000-fold, then it would not be the case that a similar shift of the ID_{50} would make model predictions generally consistent with

CDC estimates regardless as to choice of dose-response model. For the present, the uncertainty in the magnitude of the ID_{50} under conditions of general population exposure was not fully evaluated.

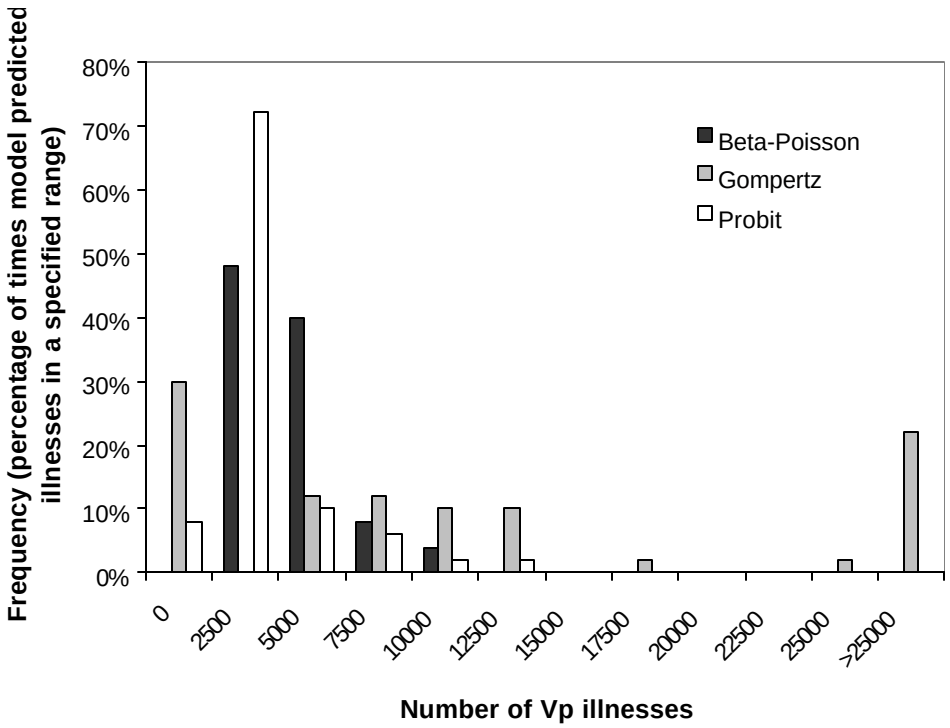

Figure VI-2. Effect of structural uncertainty of dose-response on projected number of illnesses associated with *V. parahaemolyticus* (Vp) consumption in the Louisiana Gulf Coast, summer harvest).

For example, as shown in Figure VI-2, the expected number of illnesses (i.e., mean of the distribution) associated with summer harvest in the Louisiana Gulf Coast, predicted by the Gompertz, is approximately 3,300 which is slightly higher than the expected 2,400 cases predicted by the Beta-Poisson. The expected number of illnesses predicted by the Probit model is 270, which is considerably less than that of the other two models. The results indicate that a 10-fold increase of the ID_{50} was sufficient to make model predictions consistent with CDC estimates for either the Gompertz or Beta-Poisson dose-response models. A larger shift of the ID_{50} would be necessary to predict the same number of illnesses based on the Probit dose-response. Also, as evident in the figure, there is considerable variation about the expected values; particularly for the Gompertz model, which is more heavily skewed towards higher values. This variation represents the "statistical" uncertainty of the dose-response parameters under each model (i.e., distribution of bootstrap parameter estimates) as well as the effect of other influential variability parameters, particularly the distribution of water temperature. Figure VI-2 suggests that, congruent with Figure V-4, there is considerably more statistical uncertainty associated with the Gompertz dose response than the other two dose-response models.

The distribution of probable number of illnesses predicted by the Beta-Poisson model for the whole Gulf Coast, Pacific Northwest and Mid-Atlantic regions are shown in Figures VI-3 through VI-6. Clearly, the largest numbers of projected illnesses were attributed to the Gulf Coast harvest. For the Gulf, the average number of illnesses projected to occur associated with current levels of consumption is 25 during the winter; 1,200 during the spring; 3,000 during the summer, and 400 during the fall. The spread of the distribution is approximately 2-fold. For the Pacific, the average number of illnesses projected to occur is approximately 15 during the spring and 50 during the summer. The variance of the projected number of illnesses for the Pacific during the summer is comparable to that projected for the Gulf. This similarity is primarily a consequence of the assumptions concerning the year-to-year variation of percentage of total *V. parahaemolyticus* which are pathogenic. As discussed in the Harvest Module section, it has been assumed that the extent of year-to-year variation of this parameter relative to the mean is the same in the Pacific as in the Gulf.

Model-predicted illness associated with the Pacific Coast harvest during the spring and summer, predictions are low relative to epidemiologically based estimates. From 1990 to 1996 an average of 8 culture-confirmed cases were reported in Washington State during the summer months *(72).* This statistic includes recent El Nino years that are not typical but it does not include confirmed cases in other Pacific Coast states, or the outbreaks in 1997 and 1998. Consequently, in a typical summer season at least 8 culture-confirmed cases are expected in the Pacific Northwest. This corresponds to about 160 cases assuming that illnesses are culture-confirmed at a rate of 5%. Consequently the average model-based prediction (50 illnesses) is 3-fold lower than the estimate based on the epidemiology. With regard to this discrepancy, it is possible that intertidal exposure of oysters to ambient air temperatures may have an appreciable effect on total and pathogenic *V. parahaemolyticus* densities in the Pacific growing areas *(56)*. This phenomenon is not reflected in the present risk assessment where *V. parahaemolyticus* densities at harvest are predicted based on water temperature alone. It is also possible that pathogenic strains in the Pacific (urease positive) are somewhat more virulent than those strains indigenous to the other regions of the country.

In comparison to the relatively large number of illness projected for the Gulf harvests, expected number of illnesses projected for the Mid-Atlantic harvest are 10 during the spring and 12 during the summer. As indicated in Figure VI-6, the occurrence of 30 or more illnesses associated with Mid-Atlantic summer harvest is predicted to be a relatively rare event. Expected numbers of illness likely to occur due to current levels of consumption of Northeast Atlantic oysters are estimated to be 12 during the spring, 30 during the summer, and 7 during the fall. For the winter, the distribution of probable number of illnesses for Northeast Atlantic oysters is below the resolution of the simulation based on simulation of 100,000 servings. Taking into consideration the NMFS landings data *(104)*, and then assuming 50% consumed raw plus average serving size of 12, in the Northeast Atlantic region, many more oysters are consumed in the fall (2.2 million oyster servings) vs. the spring (400,000 oyster servings). Consequently, the predicted mean number of illnesses is more in the fall than in the spring for this region in consideration of the number of servings estimated.

The low numbers of projected illnesses due to Northeast Atlantic and Mid-Atlantic oysters is attributable to both the colder water temperatures and the relatively modest harvest from these

regions during the warm summer months. Considering the extent of underreporting of illness that is likely to occur for self-limiting gastroenteritis due to *V. parahaemolyticus* infection, these low numbers of predicted illness appear to be generally consistent with the infrequency of culture-confirmed illness associated with oysters harvested from these regions. Underreporting and infrequency of culture-confirmed illness may be partly caused by the unfamiliarity of consumers and healthcare practitioners with *Vibrio*- associated illnesses, and lack of expertise in diagnosis and in laboratory detection *(34)*.

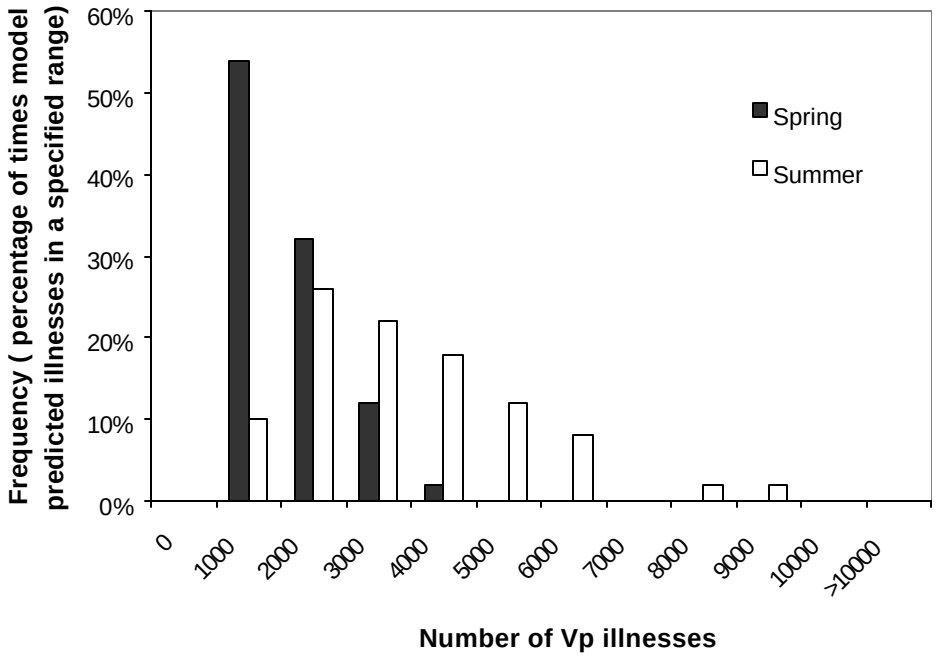

Figure VI-3. Probable number of *V. parahaemolyticus* (Vp) illnesses associated with spring and summer Gulf Coast harvests.

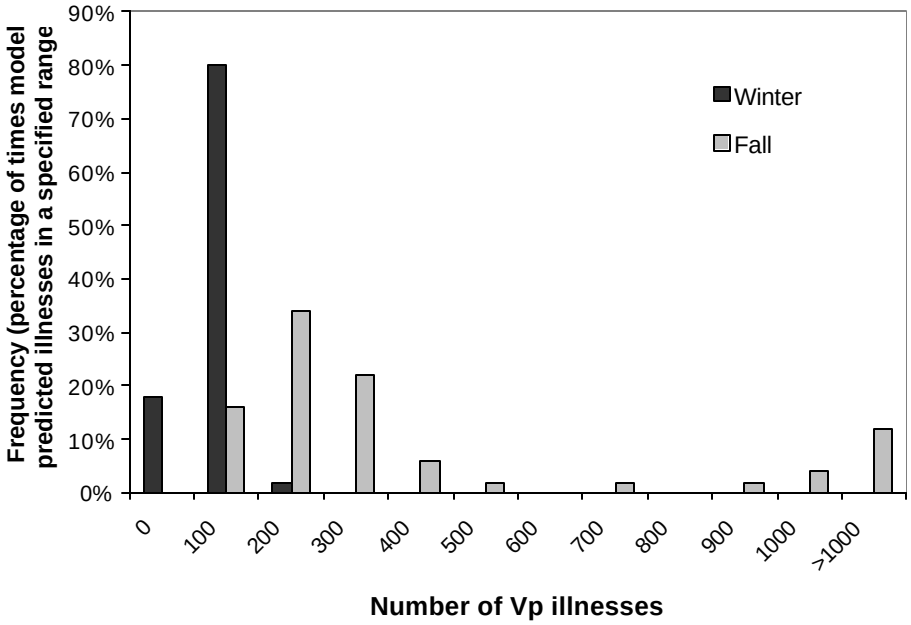

Figure VI-4. Probable number of *V. parahaemolyticus* (Vp) illnesses associated with fall and winter Gulf Coast harvests.

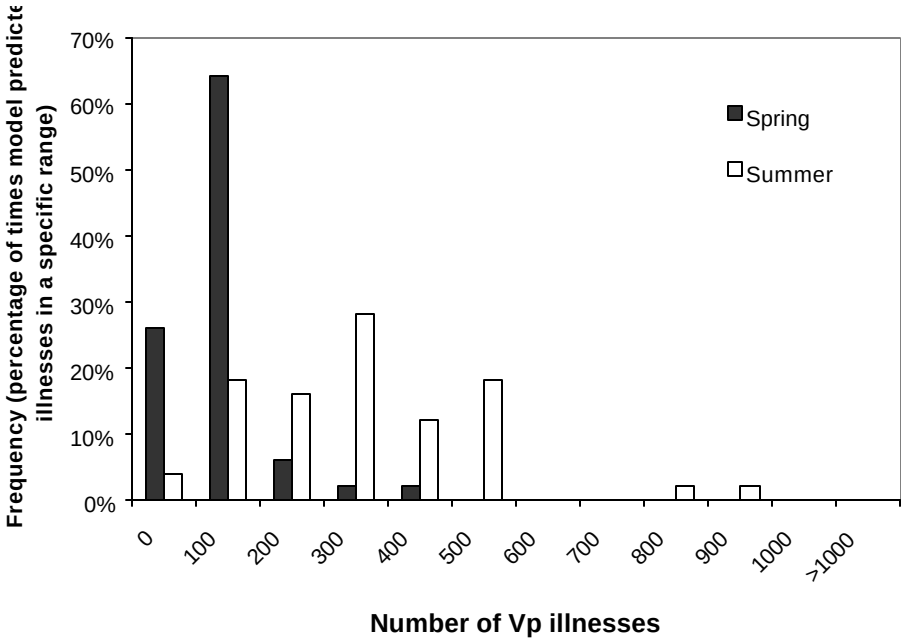

Figure VI-5. Probable number of *V. parahaemolyticus* (Vp) illnesses associated with spring and summer Pacific Coast harvest.

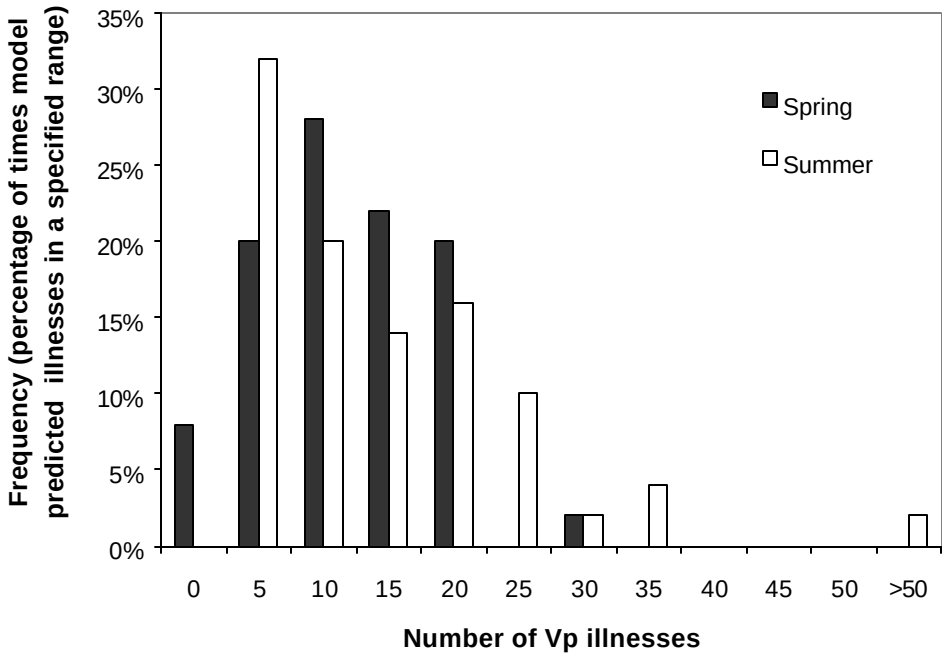

Figure VI-6. Probable number of *V. parahaemolyticus* (Vp) illnesses associated with spring and summer Mid-Atlantic harvest.

Based on the assumptions outlined previously with regard to description of the Public Health Module, the projected distribution of probable number of cases with septicemia which may occur in any given year is shown in Figure VI-7. Most of these cases are predicted to be associated with Gulf Coast oyster harvest with few cases due to Pacific Northwest harvest. Although the most probable number of cases of septicemia per year associated with ingestion of *V. parahaemolyticus*, is 4, the overall mean of the distribution is 6 cases per year, based on a sample of 50 replicated simulations. As evident in the figure, the occurrence of 15 or more cases of septicemia in a single year for the entire country is projected to be an infrequent event (i.e., an event with probability less than 0.1).

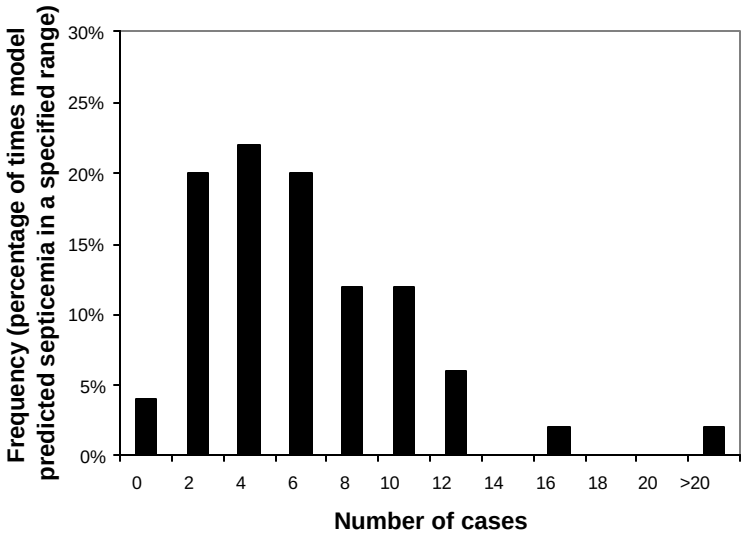

Figure VI-7. Distribution of probable number of cases of *V. parahaemolyticus*-associated cases of septicemia occurring per year (all seasons and regions).

Predicted effect of mitigation strategies on risk/probability of illness

The effect of three Post Harvest mitigations was evaluated in the simulation: (a) mild heat treatment (5 min at 50^{o} C), (b) freezing (-30^{o} C), and (c) rapid cooling immediately following harvest (e.g., aboard ship). As discussed in the Post Harvest Module, the effect of mild heat treatment has been shown to reduce the density of *V. parahaemolyticus* to nondetectable levels (at least a 4.5 $\log_{10}$ reduction) and freezing at –30^{o} C has been shown to reduce the density by approximately 2 logs.

All three potential mitigation strategies have a substantial effect on the distribution of probable number of illnesses. The effect of these mitigations was evaluated under the assumption of the Beta-Poisson dose-response model. For the Gulf Coast summer harvest (Figure VI-8), a shift in the distribution of probable number of illnesses down from a mean of 3,000 illnesses to approximately 240 illnesses is predicted under the mitigation of rapid cooling. The mean number of illnesses projected to occur under the freezing mitigation is approximately 15. As evident in Figure VI-8, the variance of the predicted distribution is also reduced under these mitigations.

The effect of mild heat treatment was found to reduce the mean risk of illness per serving to substantially less than 1 in 100,000. As discussed previously, practicalities of performing the Monte Carlo simulation necessitated limiting the simulations to 100,000 servings per season/region combination and then projecting to an estimated number of servings. Consequently, the distribution of probable number of illnesses under mild heat treatment

mitigation could not be accurately determined. For the Gulf Coast summer harvest the mean of the distribution is certainly less than 10 illnesses.

With the exception of rapid cooling, the effect of these potential mitigations on the number of illnesses is similar for other regions and seasons. The relative effectiveness of rapid cooling in the Pacific Northwest is predicted to be much less than in the Gulf Coast or Mid-Atlantic regions due to cooler air temperatures and a shorter duration of harvest. The effects of the mitigations on the mean risk per serving are shown in Figures VI-9 through VI-11 for the Pacific, Mid-Atlantic and Gulf Coast harvest for all seasons. As evident in these figures, the effectiveness of mitigation is more pronounced during the summer since the potential for growth is much greater during this time of the year.

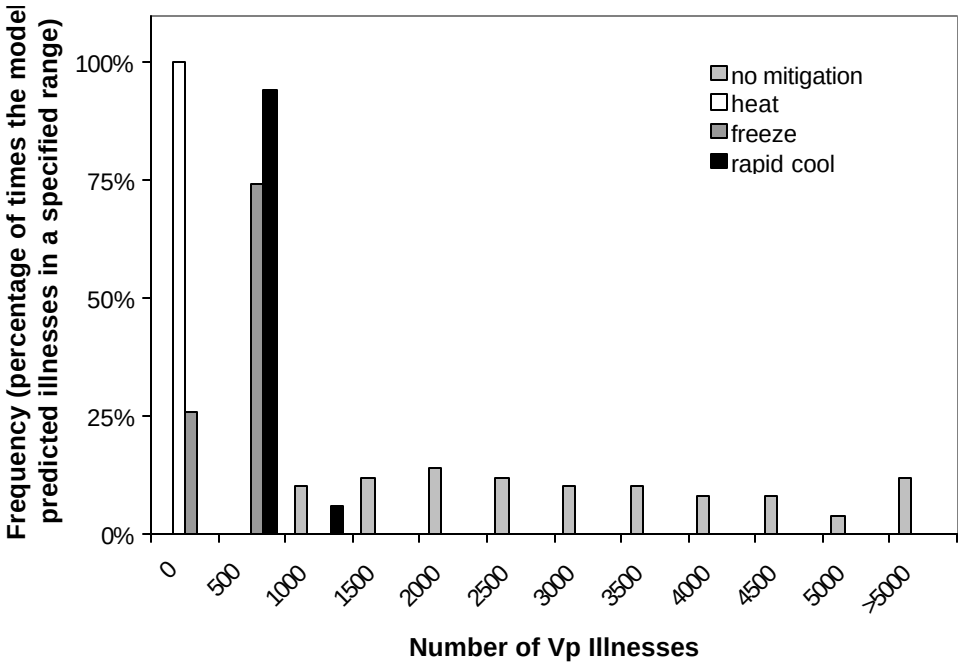

Figure VI-8. Effect of potential mitigations on the distribution of probable number of *illnesses* associated with *V. parahaemolyticus* (Vp) in oysters harvested from the Gulf Coast in the summer.

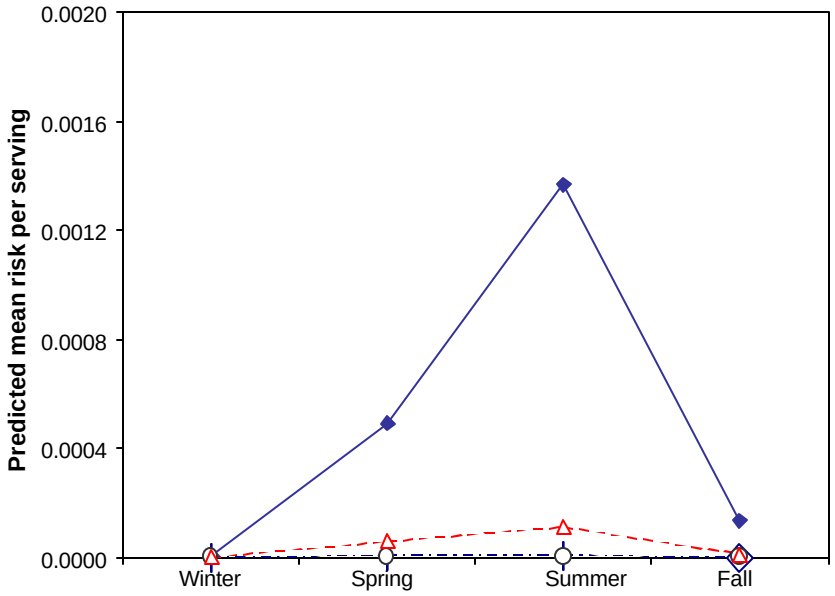

Figure VI-9. Effect of potential mitigations on the distribution mean risk of *V. parahaemolyticus* illnesses per serving associated with Gulf Coast harvest. No mitigation (¯); freezing (à); heat treatment (○); rapid cooling (△).

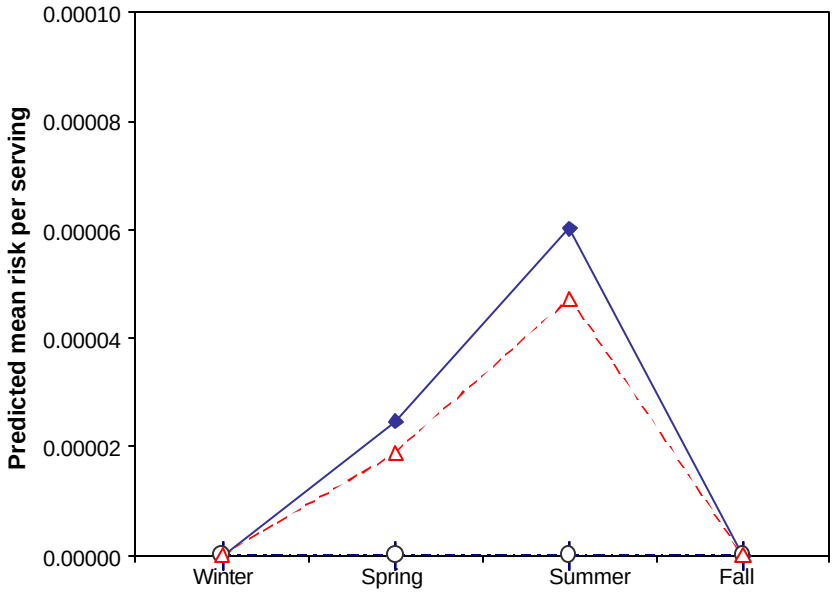

Figure VI-10. Effect of potential mitigations on the distribution mean risk of *V. parahaemolyticus* illnesses per serving associated with Pacific Coast harvest. No mitigation (¯); freezing (à); heat treatment (○); rapid cooling (△).

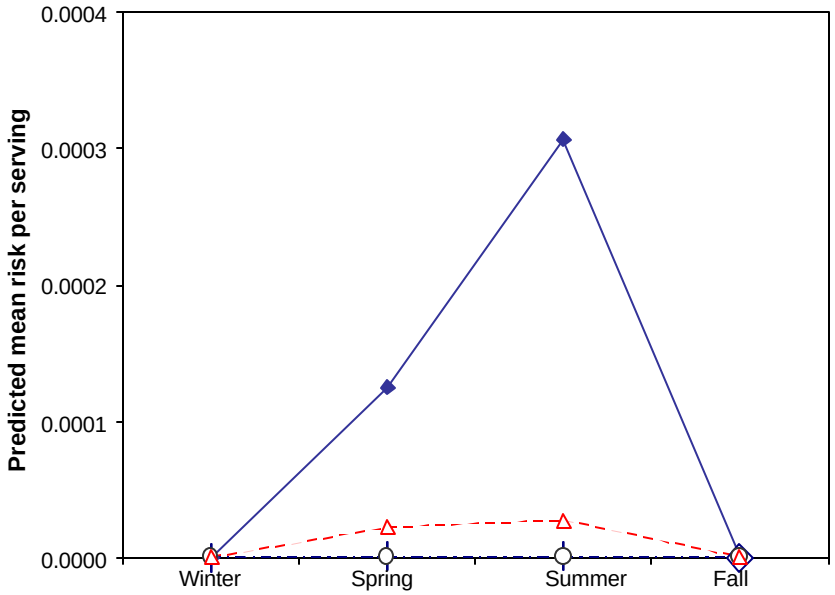

Figure VI-11. Effect of potential mitigations on the distribution mean risk of *V. parahaemolyticus* illnesses per serving associated with Mid-Atlantic Coast harvest: No mitigation (¯); freezing (à); heat treatment (○); rapid cooling (△).

Evaluation of the FDA guideline of 10,000 *V. parahaemolyticus*/g of shellfish

FDA had previously indicated that *V. parahaemolyticus* in shellfish should not exceed a level of 10,000 viable cells per gram *(64)*. The 1999 *V. parahaemolyticus* Interim Control Plan adopted by the ISSC includes the 10,000 viable cells per gram guidance. In areas where levels of greater than 10,000 cells/g oyster tissue are found, the area would need to be resampled. While the critical cause of illness in humans is the level of pathogenic *V. parahaemolyticus*, the total *V. parahaemolyticus* is used as a convenient surrogate indicator of higher risk of illness. The risk assessment cannot critically evaluate the control plan because, as the model is constructed, there is no mechanism included to account for the possibility of persistence of *V. parahaemolyticus* in specific oyster harvesting areas. Moreover, the rapidity or sensitivity of tests performed by individual laboratories for detection of *V. parahaemolyticus* is not well determined. Both of these factors are critical in evaluating the control plan.

The risk assessment does, however, allow us to ask what would be the predicted impact on the incidence of disease if we were able to exclude oysters at the time of harvest that had a specified level of *V. parahaemolyticus*. This also includes estimating the impact of what excluding oysters that had a specified level of *V. parahaemolyticus* would have on the percentage of oysters that would no longer be available. The impact of such a criterion was evaluated using parameters from the Gulf summer (Louisiana) region/season parameters. A total of fifty simulations of 33,333 iterations (Monte Carlo samples) were run individually.

The results were sorted by whether or not they caused illness and then the initial *V. parahaemolyticus* levels in the environment (i.e., at harvest) were sorted into "bins" of half log intervals. The proportion of illness associated with each half-log interval of initial *V. parahaemolyticus* level was calculated and the results were then used to estimate the potential effect of various guidance levels for "at harvest" densities on reduction of illness and associated cost in terms of percentage of total harvest lost (e.g., diverted from raw consumption market).

The results are shown in Figure VI-12 for the 10,000 per g standard, with levels of 100, 1,000 and 100,000/g included for comparison. An "at harvest" perspective was adopted here due to the fact that the current guidance level of 10,000 viable cells per g included in the 1999 *V. parahaemolyticus* Interim Control Plan pertains to oyster samples obtained at time of harvest. Although the guidance level of 10,000 viable cells per g may apply to shellstock at any time post harvest, current monitoring efforts are generally directed towards "at harvest" samples. Consequently, the potential effects of different guidance levels for monitoring of shellstock at the wholesale or retail level was not evaluated.

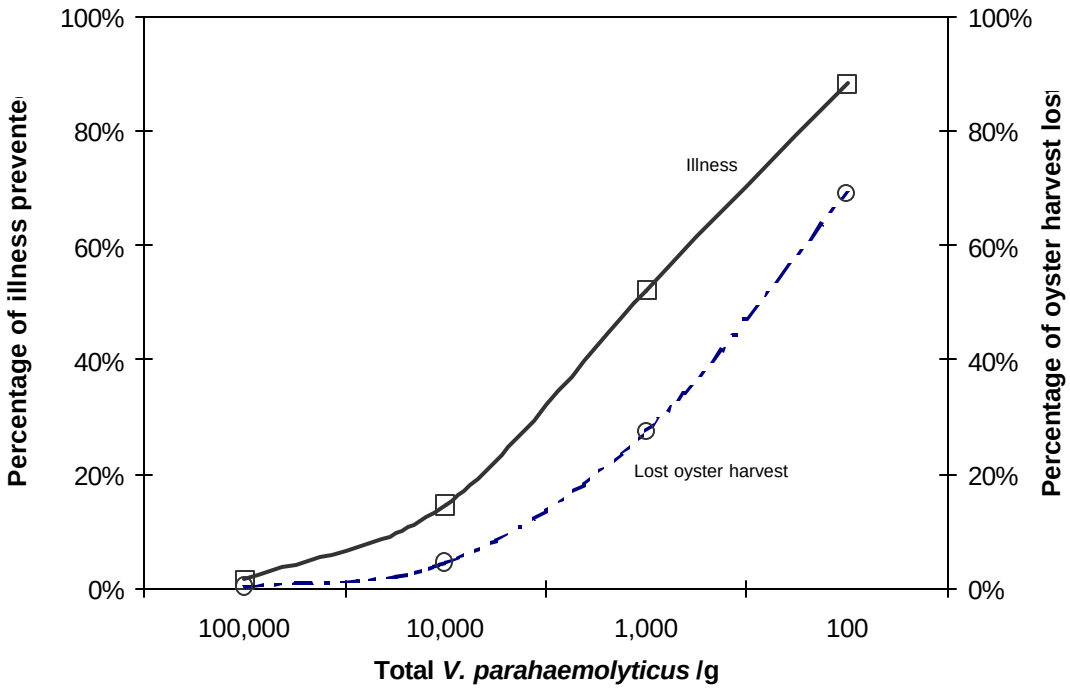

Figure VI-12. Potential effect of control of total *V. parahaemolyticus* per gram at harvest (Louisiana Gulf Coast summer harvest).

On average, the simulation results suggest that 15% of the illnesses are associated with the consumption of oysters for this region/season combination that contain greater than 4 $\log_{10}$ (10^4) *V. parahaemolyticus* per g at time of harvest. The corresponding fraction of the harvest

containing greater than 4 $\log_{10}$ (10^4) per g was 5%. Therefore, if all shellstock could be evaluated for total *V. parahaemolyticus* at time of harvest, the simulation results suggest that excluding all oysters that had levels of 10,000 viable cells per g would reduce (sporadic) illness by 15% at a loss of 5% of the total harvest from the raw consumption market. This relatively low (potential) reduction of illness is attributable to the large proportion of the harvest that would remain with a lower, but still significant, associated level of risk. In comparison, the simulation results suggest that in the absence of subsequent post harvest mitigations, "at harvest" guidance levels of 5 log (10^5), 3 log (10^3) and 2 log (10^2) total *V. parahaemolyticus* per g could (potentially) reduce the illness rate by 2%, 50% and 90% with corresponding losses of 0.3%, 25% and 70% of the harvest, respectively.

Sensitivity Analysis

A tornado plot is a convenient way of describing the factors in our model that most affect the results. The plot is called a "tornado plot" because of the similarity of the image of a tornado with the graphical arrangement of the factors from most influential at the top to least influential at the bottom. In our model, we see that the most influential factor driving the results for all the harvesting areas during the summer (Figures VI-13, VI-14, VI-15, VI-16) is the levels of *V. parahaemolyticus*. Because our model assumes that the rate of increase of pathogenic *V. parahaemolyticus*, is the same as that for total *V. parahaemolyticus*, then *V. parahaemolyticus*-associated illness results when the level of *V. parahaemolyticus* increases, thus increasing the levels of pathogenic *V. parahaemolyticus*.

In the Gulf Coast, both Louisiana and the remaining Gulf Coast regions, where the temperatures are the warmest compared to the other regions, time to refrigeration was determined by sensitivity analysis to be the second most important effect on occurrence of illness (Figures VI-13, VI-14).

The other factors analyzed have significant effects, but to a lesser extent. As one would expect, the more oysters one eats, the more likely it is that one will become ill. Also, unsurprisingly, conditions that allow for the *V. parahaemolyticus* to grow within the oyster (length of time oysters are unrefrigerated, time it takes to cooldown the oysters, water and air temperature) increase the risk of illness. Since the levels of *V. parahaemolyticus* decrease during cold storage, the length of time the oysters are refrigerated is negatively correlated with the risk and the factor points on the tornado plot in the opposite direction.

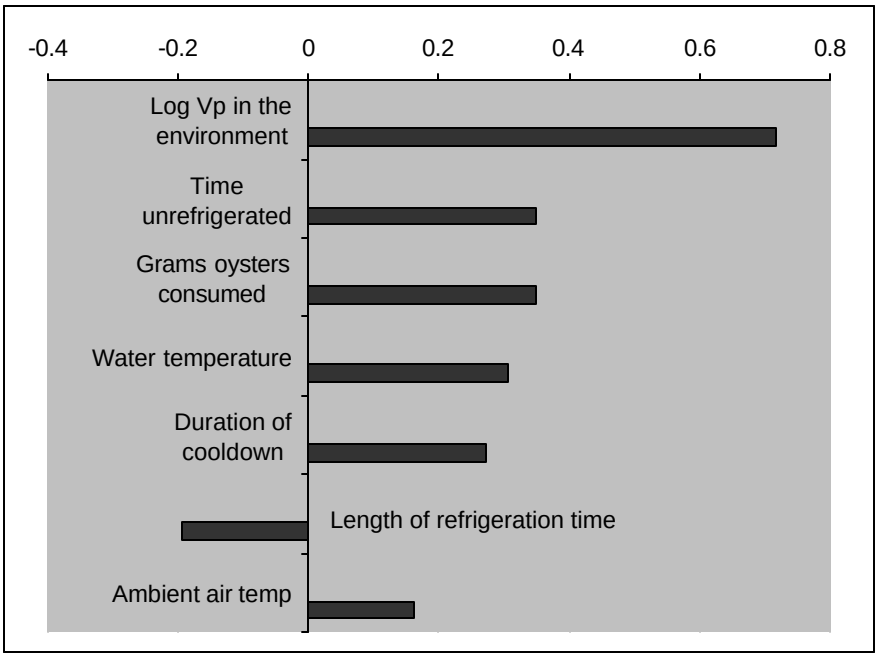

Figure VI-13. Tornado plot of influential parameters on $\log_{10}$ risk of *V. parahaemolyticus* (Vp) illness per serving of raw oysters (Gulf Coast excluding Louisiana, summer oyster harvest).

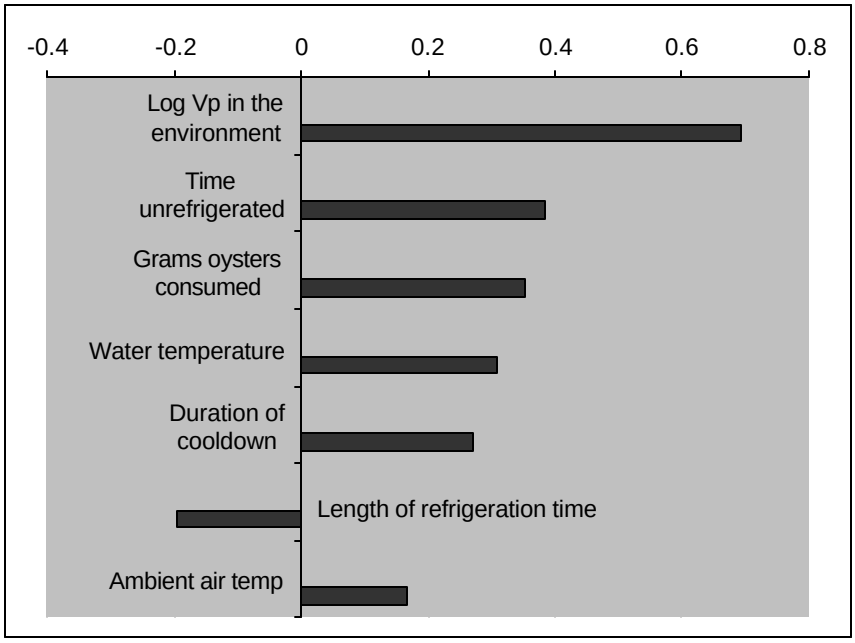

Figure VI-14. Tornado plot of influential parameters on $\log_{10}$ risk of *V. parahaemolyticus* (Vp) illness per serving of raw oysters (Louisiana Gulf Coast summer harvest).

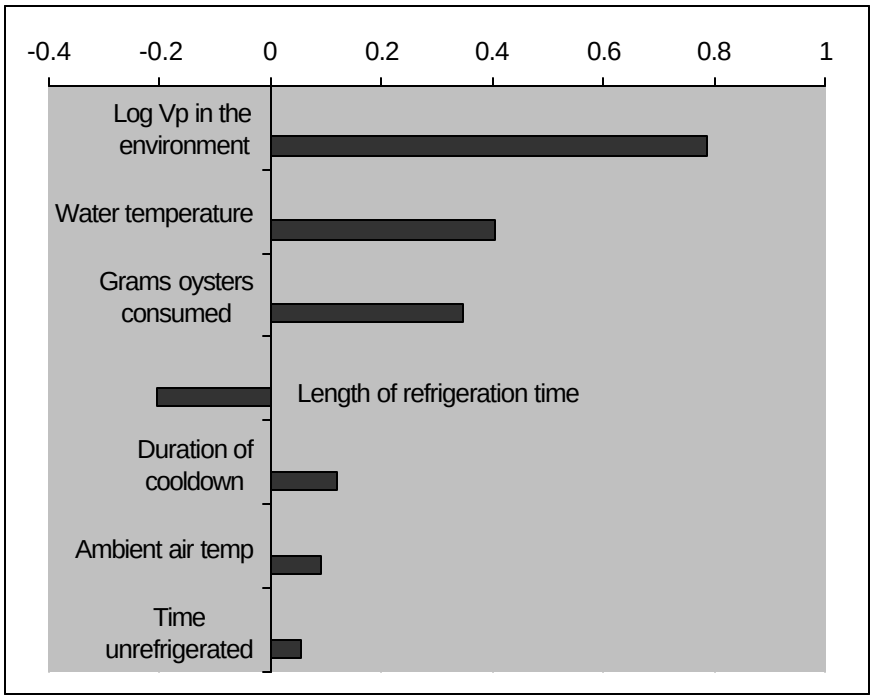

Figure VI-15. Tornado plot of influential parameters on $\log_{10}$ risk of *V. parahaemolyticus* (Vp) illness per serving of raw oysters (Pacific Northwest Coast summer harvest).

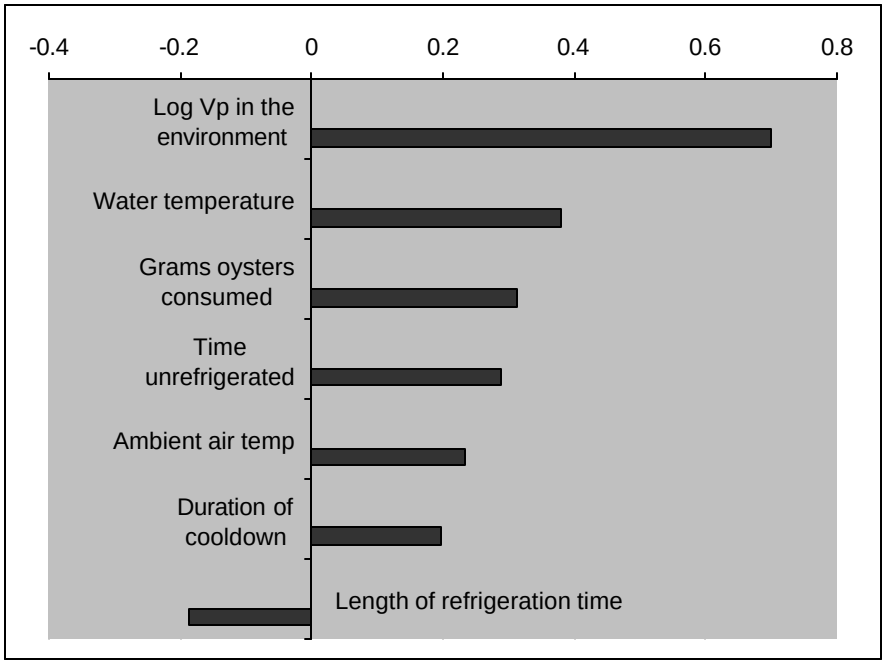

Figure VI-16. Tornado plot of influential parameters on $\log_{10}$ risk of *V. parahaemolyticus* (Vp) illness per serving (Mid-Atlantic summer harvest).

Model Validation

Reasonableness of model predictions and the appropriateness of the modeling assumptions that have been used in the risk assessment, can be evaluated by comparing model output to relevant data that were not used to develop the relationships and distributions of parameters in the model *per se*. With regard to the prediction of illness there is no independent data available for this purpose. As indicated previously, with due consideration of estimated levels of pathogenic *V. parahaemolyticus* at time of consumption and oyster landing statistics, the dose-response under conditions of feeding trials is not consistent with CDC estimates of annual illness. Consequently, the epidemiological data were used to adjust the dose-response from the conditions of human feeding trials to conditions of population exposure and do not constitute a point of validation of the model. Independent data are available on the levels of total *V. parahaemolyticus* at retail and these data have been compared to model predictions in order to assess the appropriateness of the model with respect to the Harvest and Post Harvest Modules.

A collaborative nationwide survey of *V. parahaemolyticus* densities in oysters at the retail level i.e., restaurants, oyster bars, wholesalers, etc., was conducted by the ISSC and FDA in 1998 and 1999 *(44)*. A total of 370 oyster samples were collected during the study and the harvest state was identified for all samples. This study provides the most comprehensive information available on seasonal and regional differences in density of total *V. parahaemolyticus* at time of consumption. In particular, this information provides a point of empirical validation of the assumptions used in the Post Harvest Module to predict the extent of growth that occurs post harvest. To facilitate this comparison, simulations of the distribution of total *V. parahaemolyticus* densities were carried forward through the simulation in addition to the distribution of pathogenic *V. parahaemolyticus.*

In the ISSC/FDA study, *V. parahaemolyticus* densities were enumerated by an MPN method. A relatively high proportion of the non-Gulf Coast samples had nondetectable levels. To appropriately adjust for the varying proportion of nondetectable *V. parahaemolyticus* across the different regions and seasons, estimated means were obtained by fitting a Tobit regression to the complete data set (n=370) with different harvest region and season combinations as a predictor variable. The variance about the group means was assumed to be homogeneous i.e., the same across different regions and seasons. The limit of detection varied somewhat from sample to sample but was generally 0.18 MPN/g. Clearly, estimates of regional/seasonal means near or below this threshold are an indication that a high proportion of samples from that particular grouping were not detectable and the estimate of the mean is strongly influenced by the assumptions underlying the Tobit model. In particular, estimates for the Pacific Coast were poor due to lower levels of *V. parahaemolyticus* in that region year round and the low number of samples obtained from the West Coast during the study.

Comparison of estimates of mean and population standard deviation of $\log_{10}$ total *V. parahaemolyticus* densities based on the ISSC/FDA study versus model predictions are shown in Figures VI-17 through VI-19 for the Gulf Coast, Pacific Northwest, and Mid-Atlantic harvest regions. In so far as the mean and standard deviation of the $\log_{10}$ densities predicted by simulation varies from year to year due to environmental conditions, model predictions are

presented after averaging out year-to-year variation. A certain degree of deviation from ISSC/FDA estimates is to be expected in this comparison since the empirical data were obtained over the period of a single calendar year.

Generally, the estimates of the means based on ISSC/FDA data compared well with those predicted by the simulations. In particular, model predictions of mean $\log_{10}$ densities are in good agreement with ISSC/FDA data for both the Gulf and Mid-Atlantic regions during the summer when the risk of illness is highest. For the Gulf Coast, model predictions of mean $\log_{10}$ densities in the fall are somewhat lower than those obtained by the ISSC/FDA study. With regard to this discrepancy, water temperature measurements indicate that the fall season of 1998, corresponding to the time of ISSC/FDA sampling, was somewhat warmer than usual. The NBDC buoy at Dauphin Island has been disabled since September 1998; however, the average daily noontime water temperatures in nearby Weeks Bay AL (an NERR site) during the fall of 1998 was 22.5º C, compared with a typical average of 18º C at Dauphin Island. A difference of 4.5º C corresponds to an average of 0.50 $\log_{10}$ higher density of total *V. parahaemolyticus* at time of harvest. Furthermore, warmer air temperatures would entail more post harvest growth. From January 1999 through September 1999, corresponding to the remaining period of ISSC/FDA sampling and the other seasonal comparisons, water temperatures in Weeks Bay did not differ greatly from the overall averages measured at the Dauphin Island buoy (i.e., ~1º C difference).

For the Pacific Northwest harvest, average model predictions were higher than the estimates based on ISSC/FDA data. The difference in the summer appeared to be in the range of what can be expected due to year-to-year fluctuations of environmental conditions. However, model predictions during the spring are much higher than those based on the ISSC/FDA data. A possible explanation for this discrepancy is the lack of precision associated with the estimate based on ISSC/FDA data. The number of samples on which the estimate is based was very small and consequently, due to the generally low levels of total *V. parahaemolyticus* in the spring season, the estimate of the mean is poor. Estimates could not be obtained for the Pacific winter or fall season. Similar results were obtained when considering Northeast Atlantic harvest (data not shown). Model predictions of mean density were consistent with the ISSC/FDA estimate for the summer but 1 to 1.5 $\log_{10}$ higher during other seasons of the year.

The common population standard deviation about regional and seasonal mean densities was estimated to be 1.5 $\log_{10}$ based on the ISSC/FDA data. This compares well with model predictions of the spread of the distribution when allowing for the fact that variance of measurements obtained in the survey are also a reflection of method error which has been adjusted for in the simulation. The error bars in Figures VI-15 through VI-17 denote one standard deviation above and below the mean. The interval is generally larger for the ISSC/FDA retail study data than for the simulation output.

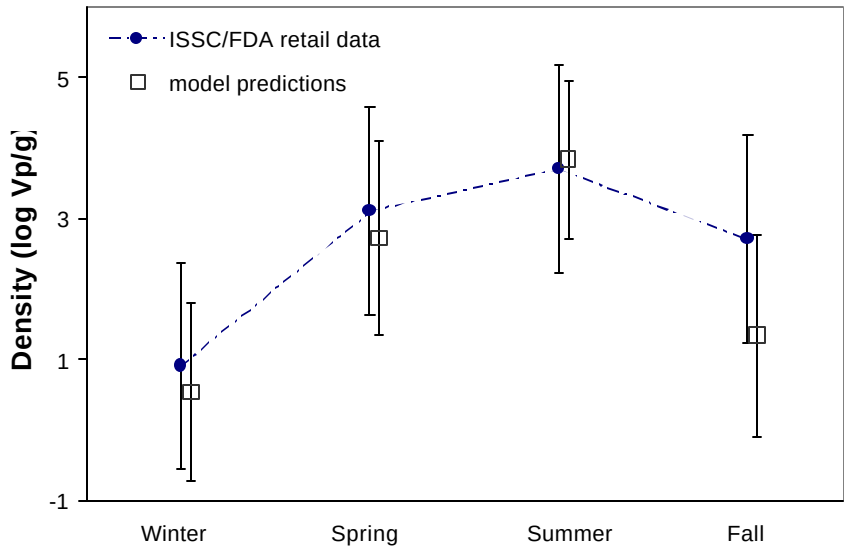

Figure VI-17. Observed retail level distribution of density of total *V. parahaemolyticus* (Vp) compared to model predictions for all seasons (Gulf harvest) *(28)*.

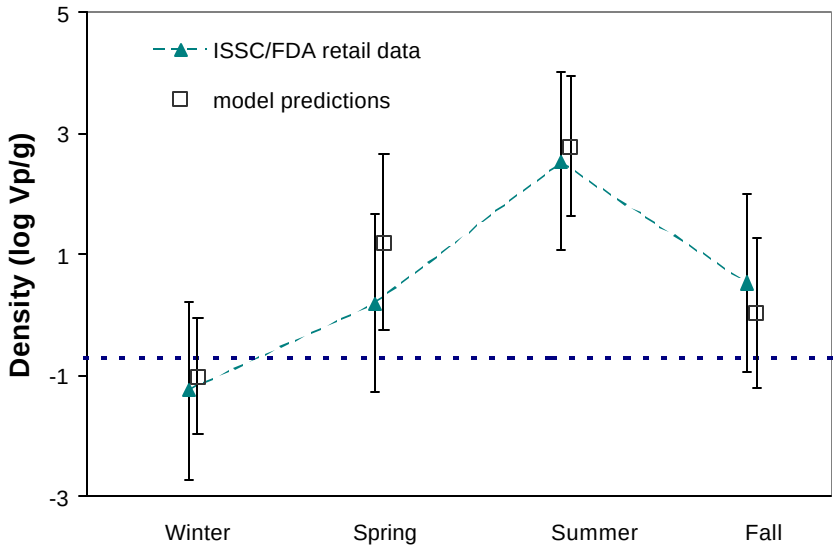

Figure VI-18. Observed retail level distribution of density of total *V. parahaemolyticus* (Vp) compared to model predictions for all seasons (Mid-Atlantic harvest) *(28)*.

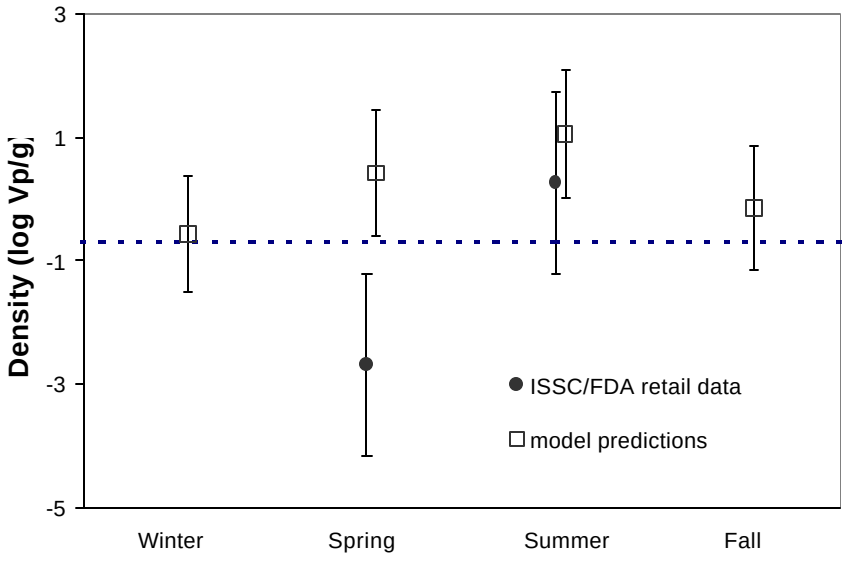

Figure VI-19. Observed retail level distribution of density of total *V. parahaemolyticus* (Vp) compared to model predictions for all seasons (Pacific harvest) ***(44)*****.**

The value of information which could be obtained by additional studies

Additional simulations were performed to examine the effect of uncertainty and variability parameters on the variance of the distribution of probable number of illnesses obtained by simulation. These simulations were directed towards determining the influence of three parameters: (a) relative growth rate of *V. parahaemolyticus* in oysters versus broth model (axenic rate); (b) combination of variability and uncertainty in the overall percentage of total *V. parahaemolyticus* that are pathogenic (% pathogenic); and (c) variation of water temperature.

The average risk per serving for Gulf Coast summer harvest under a series of five alternative assumptions is shown in Table VI-1. Fifty repeated simulations were performed under each set of assumptions. In two of the series, the parameters were either varied according to their distributions as specified in the description of the risk assessment or they were held constant at their mean values ("vary all" and "hold all"). In the remaining three series of simulations one parameter was held fixed while the other parameters varied according to their specified distributions.

Table VI-1. Effect of selected uncertainty and variability parameters with respect to Gulf Coast summer harvest: average and relative variation of predicted risk per serving for the *V. parahaemolyticus* risk assessment model

	Vary all[b]	Hold axenic (4.0)	Hold % Vp_{path} (0.2%)	Hold temp (μ=28.9, σ=1.5)	Vary None (hold all)
Average risk per serving	0.00134	0.00150	0.00160	0.00163	0.00162
Coefficient of variation [a]	98.0%	72.2%	86.0%	75.9%	53.7%

[a] standard deviation divided by the mean

[b] baseline (vary all parameters), no growth rate uncertainty (hold axenic), average percentage pathogenic known (hold % path), no temperature variation (hold temp), no uncertainty/variation in all three parameters (vary none)

Due to differences in means for the five series of assumptions, the variation in the model output (risk per serving) is summarized in Table VI-1 by the coefficient of variation (the standard deviation of a distribution divided by its mean). A clear difference is evident between the distribution of risk per serving obtained when all parameters were varied versus that when all parameters are held fixed. The coefficient of variation is 54% when all three parameters are held fixed and 98% when all parameters are varied according to their specified distributions. Consequently, the three parameters considered here account for approximately 45% of the total variation in risk per serving associated with the Gulf summer harvest. Individually, the uncertainty in growth rate proportionality and percentage pathogenic account for 26% and 12% of the total variation, respectively. In comparison, water temperature, which is a variability parameter, accounts for 22% of the total variation. These results suggest that, of the uncertainty factors considered here, the variation in the output would be reduced the most by determining the appropriate proportionality constant between growth rate in oysters versus axenic culture ("hold axenic").

An additional and important source of uncertainty underlying the predicted distribution of illness is that associated with the dose-response extrapolation from frequency of illness in feeding trial studies to conditions of human exposure. The effect of this uncertainty has not been fully evaluated in the present risk assessment. Based on consideration of CDC illness estimates, a plausible shift in the ID_{50} was determined for which model predictions of illness were consistent with the CDC estimates. A more complete evaluation of this uncertainty could be examined as a refinement of the present risk assessment.

Comments on the model

This model incorporates similar components of other risk assessments, but has several unique aspects. This model has analyzed risk in terms of region and season. Other microbial risk assessments have only looked at aggregate yearly risk. This model is scalable in that it may be applied to finer levels of analysis as data become available. Other microbial risk assessment models must be restructured to incorporate finer levels of analyses. This model has separated

variability from uncertainty by identifying four key variables as uncertain, selecting values for these variables according to specific distributions, and inserting these values into a model simulation loop. In this manner, parameters that represent variability of the model are not mixed with parameters that are uncertain. This separation has allowed us to analyze the reduction in the overall uncertainty of the analysis that we will gain if the uncertainty of an individual variable is reduced. Other microbial risk assessments have separated variability from uncertainty; however, this risk assessment has investigated the gain in information that results from reduction in uncertainty of individual variables. We discuss each of these points in turn.

This model analyzes risk within the four seasons for five primary harvesting regions (Northeast Atlantic, Mid-Atlantic, Gulf of Mexico [divided into 2 regions], and Pacific Northwest), due to differing harvest practices and climates. We could have subdivided the analysis further; however, the limitations of acquiring the data for the next level down are such that we did our analysis at the regional level. Analyzing the regions separately allows the assessors to look at mitigations that may be tailored to specific regions and seasons. Those results may then be used in a subsequent cost benefit analysis. Other microbial risk assessments have not performed region/season analysis. This limits the range of possible mitigations that can be examined.

The model is amenable to further subdivision of locality and season because it is scalable. What we mean by scalable is that the model simulates from harvest to consumption of oysters with a specific level of *V. parahaemolyticus.* Given the existence of appropriate data, the model can simulate this process from any specific harvest location at any specified time. Our analysis (regional and seasonal) was done at the level at which we had the required data set of water and air temperatures, harvest practices, *V. parahaemolyticus* prevalence, and shellfish landing information and would give us a complete US risk assessment. We could have further refined our assessment if we had complete data for the individual states, but time and resources were limiting factors. If the data were available, the model could be applied to risk assessments at the state level, shellfish harvesting area level, or, still finer, division of shellfish location and season. New uncertainties will arise if the model is applied to harvesting areas for which the required data set is incomplete. We were not able to explore the effect of incomplete (or inaccurate) data on the results of the model at this time, but we may do analyses in the future.

We have separated variability from uncertainty in our analysis because it provides the best characterization of the available information. We distinguish between model inputs that are less well characterized because of our lack of knowledge (uncertainty) and model inputs that are heterogeneous (variable). By describing some model inputs as heterogeneous we mean that we characterize some of the model inputs as being naturally variable. For example, the water temperatures of the different regions will vary in the model according to a normal distribution with a given mean and standard deviation. At the same time, we have characterized the conversion factor between *V. parahaemolyticus* growth in axenic culture and *V. parahaemolyticus* growth in oysters as an uncertainty model input. We did this because of our lack of knowledge of the true conversion factor. With more study we could reduce our uncertainty. The result of our making the distinction between model inputs that are uncertain and model inputs that are variable is that we may analyze our model and examine the effect on output of reducing the uncertainty of each of the uncertainty variables separately. In this way we provide our risk managers with insight on which uncertainty has the greatest effect on the

final results. Risk managers may then prioritize which uncertainty to reduce first in an effort to reduce the uncertainty inherent in the risk characterization of the risk assessment.

The model can be improved. At present, the model simulates risk for a set of uncertainty factors with defined variability largely based on the relationship of *V. parahaemolyticus* levels to temperature and a random selection (within defined limits) of percent pathogenic *V. parahaemolyticus*. The model does not allow a quantitative prediction of the reduction in risk resulting from implementation of the FDA/ISSC *V. parahaemolyticus* interim control plan (adopted by the ISSC in July 1999), because we cannot model the effect on the risk from embargoing subsequent harvesting after measuring an unsafe level of *V. parahaemolyticus* in a shellfish harvesting area. For us to be able to model the plan, we have several modeling needs: first, we need to scale the model to the shellfish harvesting level. To do this we need complete data sets for the individual harvesting areas. Second, we will need sensitivity and specificity data for the virulent *V. parahaemolyticus* gene probe methodology by the individual laboratories doing the tests. Third, we will need to extend the model to account for rapidity by which pathogenic *V. parahaemolyticus* levels change in specific areas. At present the model predictions are primarily based on temperature plus a random factor for population variation. The model might be improved by tailoring the rapidity of turnover of water in the shellfish harvesting area based on levels of freshwater flows, tide changes, wind direction, and depth of harvesting area. These are all areas of planned future study.

VII. DISCUSSION

The objectives of the risk assessment were two-fold: (a) to create a mathematical model and assess the current risk of becoming ill due to the consumption of pathogenic *V. parahaemolyticus* in raw oysters; and (b) develop a comprehensive and current scientific framework, which will assist the agency with the review of current programs relating to the regulation of *V. parahaemolyticus* in raw molluscan shellfish to ensure that such programs protect the public health. The risk assessment task force was also charged to evaluate: the evidence for increased risks from specific newly emerging "outbreak strains", the effectiveness of potential strategies for limiting exposure of the public to raw molluscan shellfish, particularly oysters containing pathogenic *V. parahaemolyticus,* the current criteria for opening and closing harvest waters, and FDA's current established guideline level of 10,000 *V. parahaemolyticus*/g of food.

The risks for sporadic illnesses occurring due to *V. parahaemolyticus* in oysters are determined by this risk assessment. Assessment of the risks associated with oyster-borne outbreaks caused by pathogenic *V. parahaemolyticus* will not be feasible until a later date. The data needed to model outbreak-associated risk are not as yet available. These data will be obtained from the interim control strategy for preventing outbreaks caused by *V. parahaemolyticus* by monitoring oyster meats for strains having the TDH gene, implemented in 1999 by the Interstate Shellfish Sanitation Conference (ISSC). Monitoring for pathogenic strains and implementation of harvest controls commenced in the spring of 2000 (some states began monitoring in 1999). To reasonably assess the effectiveness of this interim control strategy, FDA needs the following information: (a) which regions are monitoring, (b) the number of growing areas monitored in each region, (c) the number of total samples collected and the number positive for *V. parahaemolyticus* in each region, (d) the sensitivity of testing, (e) oyster landing data for each site and region during periods of monitoring, and (f) case data on oyster-borne illnesses caused by pathogenic *V. parahaemolyticus.*

The solicitation and assemblage of information and scientific data on *V. parahaemolyticus* from many sources produced a thorough, up-to-date compilation. This information was used in the construction of a mathematical model to produce results on the risk of illness incurred by eating raw oysters containing pathogenic *V. parahaemolyticus.* Three basic factors were found to be associated with this pathogen and consumer risk: the level of pathogenic *V. parahaemolyticus* in seafood at harvest, effect of post harvest handling and processing, and the ability of the organism to multiply to an infective dose. As a result, the risk assessment project was divided into three separate modules, which corresponded to different stages leading potentially to consumer exposure: the Harvest, the Post Harvest, and the Public Health Modules. The Harvest Module estimated the prevalence of pathogenic *V. parahaemolyticus* at time of harvest. The Post Harvest Module determined the role of post harvest processing and handling on the levels of pathogenic *V. parahaemolyticus* at consumption. The Public Health Module estimated the risk of illness caused by this organism. Because of harvesting and temperature differences, for the purpose of the model, the United States harvest areas were divided into five regions, and each region was divided into four seasons. Differences existing in oyster harvesting practices

and climates in the United States were sufficiently significant to identify five separate geographic regions (Northeast Atlantic, Mid-Atlantic, Pacific Northwest, Louisiana Gulf Coast, and the remainder of the Gulf Coast) for each season, for consideration in modeling each of the modules. Factors influencing the risk of illness posed by *V. parahaemolyticus* were identified and incorporated in each module as appropriate. Integration of the various parameters comprising these modules into a quantitative risk assessment model has provided a more comprehensive understanding of the relative importance and interactions among these factors influencing risk. This gain in understanding should serve to facilitate several processes, including the formulation of effective guidance for the industry, regulators and consumers, the evaluations of risk mitigation strategies, and the development of options and policies for managing risk.

While providing a framework for understanding the relationship of risk to various parameters, the development of the risk assessment model necessarily required certain assumptions to fill the data gaps. The assumptions incorporated in the model were reviewed by NACMCF at a public meeting in September1999. Based on the information currently available, for the Harvest Module, it was assumed that the presence of the TDH gene be used as the basis for pathogenicity. It is not currently known what average levels of TDH-positive strains actually exist in shellfish, nationally or regionally. The estimates made in the *V. parahaemolyticus* risk assessment, based on observed frequency of TDH-positive isolates, were the best possible with the data currently available. However, since we do not know how this frequency may vary from one year to the next, we assumed a 2-fold up or down triangle distribution. Also, within a given year, we were unsure about the variance of percentage pathogenic in one composite of oysters to the next. For example, outside of the Pacific Coast, percentage pathogenic *V. parahaemolyticus* in a given year ranged from 0.1% to 0.3%; for the Pacific Northwest the range used was 2% to 4%. Furthermore, these estimates are based on older data, and may not be predictive of future years, given that frequency of percentage pathogenic *V. parahaemolyticus* may be changing as new outbreak strains emerge or reemerge, such as the emergence of O3:K6 or recurrence of known outbreak strains such as O4:K12.

For the Post Harvest Module, several assumptions were made based on the knowledge of current post harvest practices and information available. The time oysters are harvested to the time they are refrigerated was based on the current NSSP requirement*(64)* put into effect in 1997. The extent of growth that occurs during the period of time from harvest until the time that oysters are first placed under refrigeration is determined by three factors: (a) the growth rate of *V. parahaemolyticus* as a function of temperature; (b) the temperature of oyster meat after harvest and (c) the length of time held unrefrigerated. The growth rate of pathogenic *V. parahaemolyticus* in oysters was assumed to be one fourth that in broth culture at all temperatures. This rate was based on the model of Miles *et al. (95),* and the corresponding studies in oysters by Gooch *et al.* at 26^{o} C *(51)*. Also, since the *V. parahaemolyticus* organisms do not change their growth environment after harvest (within the oyster meat), it was assumed that lag time was negligible and was therefore omitted from the growth model. Regarding growth rates, preliminary studies at GCSL, showed no significant difference between pathogenic and non-pathogenic strains of *V. parahaemolyticus.* Since data on cooling rates of commercial oyster shellstock could not be located, the time for oysters to cool after being placed under refrigeration was assumed to be quite variable. This depended on efficiency of the

cooler, quantity of oysters to be cooled and their arrangement in the cooler. A uniform distribution between 1 and 10 hours was used to model this parameter based on preliminary GCSL experiments for the time it took a single shell oyster at 30°C placed into a 3° C cooler to reach that temperature, and the time it took for 24 oysters in an uninsulated plastic container at 26° C to reach 3° C.

For the sake of simplicity of the model, we assumed that consumption patterns were the same for both the sensitive and otherwise healthy population, for all regions. It was assumed that all virulent/pathogenic strains of *V. parahaemolyticus* are equally virulent with the same dose-response as those strains fed to human volunteers in earlier studies. This assumption was based on personal communication with Dr. Nishibuchi, Kyoto University *(105)*, who stated that due to lack of information, it is not known whether there are differences in virulence among different strains.

Our model clearly illustrated that air and water temperatures were the driving factor for initial pathogen loads as well as continued growth after harvesting, but there is some uncertainty due to lack of data showing correlating *V. parahaemolyticus* levels. It is also noteworthy that in the Pacific and Mid-Atlantic, the lower air temperatures reduce the importance of air temperature and time unrefrigerated compared to the Gulf Coast.

The risk assessment model illustrated that the most significant factor influencing probability of illness due to *V. parahaemolyticus* is the level of *V. parahaemolyticus* present in the oyster at harvest. However, the model is based on a strong correlation between total and pathogenic *V. parahaemolyticus* levels at time of harvest. We have also assumed that pathogenic strains of *V. parahaemolyticus* grow at the same rate as non-pathogenic strains. Consequently, as the level of total *V. parahaemolyticus* increases so does the number of pathogenic *V. parahaemolyticus*.

For the Gulf Coast, the second most influential factor for occurrence of illness is the duration that oysters are left unrefrigerated after harvest. For the remaining regions modeled, i.e., Mid-Atlantic and Pacific Northwest, water temperature was the second most influential parameter. For all regions, however, the amount of oysters consumed was the third most influential factor. It is interesting to note that time the oysters were left unrefrigerated was more significant for the Louisiana Gulf Coast than for the remaining Gulf Coast. It is known that many oyster harvesting areas are further offshore in Louisiana than in the rest of the Gulf Coast, and therefore it takes longer for the boats to return to the shore after harvest, leaving the oysters unrefrigerated for a longer time period.

Modeling of the Post Harvest Module demonstrated that if oysters are not refrigerated rapidly after harvest as recommended by NACMCF *(102)*, *V. parahaemolyticus* rapidly multiply in oysters resulting in much higher levels. The model's simulation of mitigation strategies indicated a significant reduction in the probability of illness when the oysters are cooled immediately after harvest. Furthermore, *V. parahaemolyticus* densities were shown to decrease slowly during refrigerated storage, as also stated by the MSI and PCSGA in response to the Federal Register notice Docket No. 99N-1075 *(43)*. Moreover, the use of mild heat treatment, which causes at least a 4.5 $\log_{10}$ decrease in the number of viable *V. parahaemolyticus* in

oysters, practically reduced to zero the probability of illness occurring. Freezing, which causes a 1 to 2 $\log_{10}$ decrease substantially reduced the probability of illness.

Earlier human trials conducted in Japan showed an increase in the number of illnesses with increasing levels of pathogenic *V. parahaemolyticus*. Different dose-response models were compared for the purpose of extrapolating risk of illness estimated on the basis of human feeding trials at high levels of exposure to the lower levels of exposure associated with consumption of raw oysters. However, consideration of CDC estimates of annual illness suggested that the dose-response under conditions of population exposure was different than that observed in human volunteer studies. In other words, direct extrapolation of the dose-response under conditions of exposure in the feeding trials is not supported by the epidemiological data. The human feeding trials were conducted under conditions of concurrent antacid administration. Due to possible food matrix effects of the oyster, dose-response was shifted by 1 $\log_{10}$ from that based on published clinical trials. Preliminary data have shown that this shift is "supported" by consideration of the CDC numbers of *V. parahaemolyticus* infection. Distributions of ingested dose were developed by considering the probabilistic variation of number and meat weight of oysters in a serving in addition to the expected variation of the density of pathogenic *V. parahaemolyticus* determined in the Harvest and Post Harvest Modules.

The outputs from this project provide estimates of risk for illness among consumers of raw oysters (average nationwide yearly incidence of 4,750 cases per year, with a range from 1,000 to 16,000 cases - for the Gulf Coast, 25 (winter), 1,200 (spring), 3,000 (summer), and 400 (fall); for the Pacific Northwest, 15 (spring) and 50 (summer); for the Mid-Atlantic, 10 (spring) and 12 (summer); and for the Northeast Atlantic, 12 (spring), 30 (summer) and 7 (fall)). Risks increase with increasing levels of total *V. parahaemolyticus* and therefore pathogenic strains of *V. parahaemolyticus*.

The model made it possible to develop a mathematical means of relating potential microbiological criteria with both the predicted percentage of illness prevented and the predicted percentage of the oyster landings that would no longer be available to consumers if the criterion could be implemented with 100% efficiency. Retail surveys of oysters, clinical studies and outbreak investigations, have shown that the guidance level of 10,000 viable *V. parahaemolyticus* cells/gram of oyster meat may not be relevant to safety. Simulations on the rate of illness caused by oyster-servings where the levels of *V. parahaemolyticus* at harvest are at or above 10,000 cells/g suggest that approximately 15% of the illnesses are associated with the consumption of oysters containing greater than 10,000 *V. parahaemolyticus*/g at time of harvest. This is a consequence of the fact that higher levels of pathogenic *V. parahaemolyticus* are more likely to occur with higher levels of total *V. parahaemolyticus*. Nevertheless, even at these high levels, the risk of illness per serving is still comparatively low. Comparing the number of servings that cause illness to those that don't, the simulations demonstrate that on average 0.6% of the servings result in illness when *V. parahaemolyticus* levels are at 10,000 cells/g or above.

The risk assessment team addressed the questions that it was charged with as described below.

- What is the frequency and extent of pathogenic strains in shellfish waters and shellfish?
  - There is a need for more information on virulence factor determination, such as invasion of the enterocytes *(3)*, and production of an enterotoxin *(61)*, and particularly, urease production *(77, 79)*. Since the data on levels of pathogenicity pose a large uncertainty, the model was run with a range of estimates for TDH, from 0.0032%, to 3.2%. This produced a range of possible illness rates, with the most likely being identified (along with the reasons why it seems the most likely) as the most probable level of risk.
  - The epidemiology and pathogenicity of *V. parahaemolyticus* will be better understood once the ecological nuances of the microbe-host dynamics in the estuarine ecosystem are elucidated.

- What parameters can predict presence?
  - Our model clearly illustrated that air and water temperatures were the driving factor for initial pathogen loads as well as continued growth after harvesting, although some uncertainty exists due to lack of direct measurement of the relationship in regard to pathogenic *V. parahaemolyticus* levels per se.
  - The literature has shown that other factors not incorporated into the model may also predict presence of pathogenic *V. parahaemolyticus.* These include:
    - Salinity - The Texas outbreak showed a significant increase in the salinity levels and temperature in the spring (May-June) of 1998 compared with the previous five years, however these levels are very similar to normal summer temperature and salinity levels. It is also possible that the sudden increase in temperature/salinity causes the *V. parahaemolyticus*, both the pathogenic and non-pathogenic strains to grow quickly or multiply. However, our model suggested that salinity was not, in fact, an important variable.
    - Ballast waters - Although there was insufficient data to quantitatively model this parameter, ballast waters have been associated with *Vibrio* illnesses. *(93).*
    - Other factors, which were also not quantitatively modeled, such as immune status of the oyster, have also been shown from the literature to play an important role in the prevalence of pathogenic *V. parahaemolyticus*. Oyster data from the DePaola *et al.* study *(38)* on the incidence of *V. parahaemolyticus* in U.S. coastal waters and oysters were probably representative of different physiological states of the oysters. Once sufficient data have been accumulated to actually investigate the *V. parahaemolyticus* load and health of the oysters, the information can be incorporated into the model. There is a need to correlate the number of *V. parahaemolyticus* with the percentage oysters diseased.

- How do levels at consumption compare to initial levels?
  - Preliminary analysis of the ISSC/FDA retail study showed higher *V. parahaemolyticus* densities at retail than at harvest for the Gulf Coast. Model predictions suggest that the difference between densities at harvest versus those at time of consumption is largely attributable to the extent of growth that occurs after oyster harvest is landed and has not yet been cooled to no-growth temperatures. The extent of the difference varies by region and is largest during the summer ranging from 0.50 for the Pacific Northwest to 1.25 $\log_{10}$ for the total Gulf Coast. During the spring the difference between densities at

harvest versus time of consumption is approximately 0.75 $\log_{10}$ for the Gulf Coast and somewhat less for other regions of the country. The simulation results are consistent with the *V. parahaemolyticus* densities observed at retail in the ISSC/FDA study.

- What is the role of post harvest handling?
  - The model demonstrated that if oysters are not refrigerated after harvest, *V. parahaemolyticus* rapidly multiply in oysters resulting in much higher levels. The model revealed a significant reduction in the probability of illness when the oysters are cooled immediately after harvest. Furthermore, *V. parahaemolyticus* densities are shown to decrease slowly during refrigerated storage.

- What intervention strategies can be used?
- Our simulation of post harvest mitigation strategies has shown that mild heat treatment which causes at least a 4.5 log decrease in the number of *V. parahaemolyticus* essentially eliminates any probability of illness.
- Freezing combined with frozen storage, which causes a 1 to 3 log decrease substantially reduces the probability of illness.

- What is known about dose-response?
  - Early Japanese feeding studies described in previous sections, have shown an increase in number of illnesses with increasing levels of pathogenic *V. parahaemolyticus*. However, the extent to which the estimate of the ID_{50} under conditions of human feeding trials underestimates the "true" population ID_{50} is unknown. In consideration of CDC estimates of annual illness rates, model predictions based on projected levels of pathogenic *V. parahaemolyticus* suggest that the dose-response for illness under conditions of normal population exposure may be significantly different from the conditions of the feeding trials. Possible reasons for the difference include food matrix or immunological effects of preexposure to the organism including antibodies/vaccines to the organism *(88)*.

- How does dose-response vary for different strains of *V. parahaemolyticus*?
  - Based on insufficient epidemiological data and personal communication with Prof. Mitsuaki Nishibuchi who suggested that more research is needed to determine whether strains differ in virulence *(105)*, we have assumed equal virulence for all virulent strains. The assessment of dose-response according to virulence was developed based on the organism's ability to produce TDH. This may be modified as new data become available that identify new virulence determinants. As mentioned previously, other strain characteristics, such as invasion of the enterocytes *(3)*, and production of an enterotoxin *(61)* not normally investigated in the environmental or in clinical isolates, may also be important in characterizing pathogenicity. In particular, recent data from British Columbia *(77)* may suggest an association of urease positive strains with clinical isolates, which may or may not be TDH positive.

- How does dose-response vary among humans with different susceptibilities?
- Epidemiological data indicate that the whole population is susceptible to infection.

- Differences in dose-response among humans with different susceptibilities, are not as yet known. However, given infection, regardless of dose, there is a greater probability of infection leading to more severe illness, such as septicemia and death, among the subpopulation with a concurrent underlying medical condition. As mentioned in the Risk Characterization section, using case series data provided by the CDC, diarrhea in an infected person has a 12% chance of going on to septicemia if that person belongs to the sensitive subpopulation, and *V. parahaemolyticus* has been culture-confirmed.

- Is current knowledge adequate in assessing the risk of consuming raw oysters containing pathogenic *V. parahaemolyticus*?
  - Our gain in understanding of the relative importance and interactions among the factors influencing risk should serve to facilitate several processes, including the formulation of effective guidance for the industry, regulators and consumers, the evaluations of risk mitigation strategies, and the development of options and policies for managing risk.
  - However, the risk assessment also identified data gaps, which will need to be addressed by research.

- Where should future research be directed to reduce uncertainty in risk estimate?
  - Future research should be directed at the data gaps as listed below

Data Gaps and Future Research Needs

Deficiencies of the current research with respect to risk assessment were identified in order to suggest future research or further data gathering to reduce uncertainties.

- Incidence/frequency of pathogenic *V. parahaemolyticus* in water and shellfish.
  - Factors that affect incidence of pathogenic *V. parahaemolyticus* in the environment.
  - Role of oyster physiology and immune status in levels of *V. parahaemolyticus.* There is a need to correlate the number of *V. parahaemolyticus* with the percentage oysters diseased.
  - More research on the potential virulence factors of pathogenic strains other than TDH, e.g. urease, enterotoxins. *V. parahaemolyticus* strains that do not produce TDH, TRH, or urease have recently been found to induce fluid accumulation in suckling mice and diarrhea in a ferret model after oral inoculation in a dose-dependent manner *(84).* Correlation between clinical and environmental incidence of these strains is yet to be determined.

- Growth rate of *V. parahaemolyticus* within oysters at temperatures other than 26° C; including the issue of potential differences in the growth rate of pathogenic strains versus total *V. parahaemolyticus* populations.

- Rates of hydrographic flushing (water turnover) in shellfish harvest areas based on levels of freshwater flows, tidal changes, winds, depth of harvesting area and how these factors may influence pathogenic *V. parahaemolyticus* levels.

- Dose-response data.
  - FDA is currently funding a cooperative agreement with the University of Maryland to acquire a dose-response curve for *V. parahaemolyticus* in humans after extrapolation from animal studies. Volunteers will be fed raw oysters containing *V. cholerae* non-O1 at varying doses. Animals will be fed with *V. cholerae* non-O1 and *V. parahaemolyticus* at varying doses. After the animal doses and strains have been compared, the *V. parahaemolyticus* dose-response observed in animals will be extrapolated to humans, based on the data from the clinical and animal studies with *V. cholerae* non-O1. Better dose-response data will be available within the next two years after the completion of this study.
  - More intensive investigations of shellfish foodborne disease outbreaks in such a way as to examine the relationships between the dose of contaminated food items ingested and the severity of the resulting illness controlling for host factors.

- More data from State surveillance systems.

- Consumer handling of oysters.

- Improved global public health surveillance of *V. parahaemolyticus* to identify new epidemic strains as they emerge.

- FDA is already currently involved in several collaborative efforts with the ISSC and the states, to protect the public from illness caused by *V. parahaemolyticus* and other seafood pathogens. Some of these efforts have provided invaluable information for the risk assessment. Data acquired in the future from the other efforts (listed below) will be used to validate and update our model.
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- The *V. parahaemolyticus* interim control plan for closing and reopening harvest waters was adopted by the ISSC in July, 1999, for areas confirmed as an original source of oysters harboring pathogenic (TDH+) *V. parahaemolyticus*, that have been associated with two or more confirmed illnesses within the last three years. The outcome of modeling this plan will also enable the evaluation of the criteria for closing and reopening harvest waters. When the requisite data become available, it is anticipated that the reduction in risk afforded by the ISSC prevention strategy as well as the criteria for opening and closing harvest waters can be modeled.

- Oyster harvest monitoring survey – FDA in conjunction with the ISSC and the states is conducting a study to determine the levels of *V. parahaemolyticus* in oysters in the growing areas before they are harvested. This study was initiated in 1998, and some of the data obtained thus far, was incorporated into the risk assessment when determining levels of total and pathogenic *V. parahaemolyticus*.

- The time to refrigeration data obtained from states that had had *V. vulnificus* illnesses, proved to be invaluable in providing the data for the time to temperature simulations in the Post Harvest Module.

In conclusion, this risk assessment significantly advances our ability to describe our current state of knowledge about this important foodborne pathogen, while simultaneously providing a framework for integrating and evaluating the impact of new scientific knowledge on enhancing public health.

The results of this draft risk assessment on *V. parahaemolyticus* are influenced by the assumptions and data sets that were used to develop the exposure assessment and hazard characterization. These results, particularly the predicted estimates of risk for illness among consumers of raw oysters, and the most significant parameters, which influence the incidence of illness, could change as a result of future data obtained from the Interim Control Plan and the FDA actively seeking new information, scientific opinions, or data during the public comment period. It is anticipated that periodic updates to the risk model will continue to reduce the degree of uncertainty associated with risk estimates, and that this will assist in making the best possible decisions, policies, and measures for reducing the risk posed by *V. parahaemolyticus* in raw molluscan shellfish.

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IX. APPENDICES

Appendix I

Chronology of Technical and Scientific Reviews of the FDA *Vibrio parahaemolyticus* Risk Assessment Document

We solicited the advice and opinions of State shellfish and scientific experts, and the public throughout the conduct of this *Vibrio parahaemolyticus* risk assessment. A summary of the dates, type of review activity, and participants is provided on the next page.

Chronology of Technical and Scientific Reviews of the FDA *Vibrio parahaemolyticus* Risk Assessment

Date	Activity	Participants
January 1999	Risk Assessment Team assembled	FDA
May 7, 1999	Federal Register Notice; request for comments and for scientific data and information	Public
May 27, 1999	Public meeting (Chicago, IL)	NACMCF; Public; VPRA team members
August 13, 1999	Federal Register Notice of public meeting	Public
September 24, 1999	Public meeting; request for comments on the risk assessment approach and assumptions (Washington, DC)	NACMCF; Public; VPRA team members
December 1999	Request for scientific review of draft risk assessment document	RAC members
December 1999	Technical discussion of the draft risk assessment document	RAC annual meeting (closed)
December 1999	Intensive review of model	Dr. David Gaylor, FDA/NCTR
March 31, 2000	Internal scientific review of draft document	Selected FDA risk managers
May 29, 2000	Technical review of document	Selected government experts and SGE's
May 29, 2000	Review of model and mathematics	Selected government experts and SGE's
July 28, 2000	Internal scientific review of draft document	Selected FDA risk managers
August 4, 2000	Presentation on update of *V. parahaemolyticus* risk assessment at the IAFP meeting, Atlanta, GA	IAFP attendees

NACMCF - the National Advisory Committee on Microbiological Criteria for Foods.
RAC - the U.S. government Interagency Risk Assessment Consortium
SGE - Special Government Employees
IAFP - International Association for Food Protection

Appendix II.

Model for the FDA *Vibrio parahaemolyticus* Risk Assessment

Electronic copy available: http://www.foodsafety.gov/~dms/fs-toc.html