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CCDEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

NATIONAL ADVISORY COMMITTEE

ON

MICROBIOLOGICAL CRITERIA FOR FOODS

VOLUME IV

Friday, September 24, 1999

8:05 a.m.

Washington Plaza Hotel
10 Thomas Circle
Massachusetts Avenue & 14th Street
Washington, D.C. 20005

MILLER REPORTING COMPANY, INC.
507 C Street, N.E.
Washington, D.C. 20002
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P R O C E E D I N G S

CHAIRPERSON WACHSMUTH: Welcome. Good morning. It's a nice day out there. It's a nice day in here. I'm glad to see you all.

This is the last day of our four-day session, and we will have the Vibrio parahaemolyticus risk assessment first. Then, after the general discussion of that, we'll decide, based on the time, whether we might again look at the recommendations on bare-hand contact. We will try to do that today in this session. If not, we may finish it up by mail later. We'll just see where we are at lunch and decide then.

And then in the afternoon, a couple of small--we hope shorter--updates on the small plant hazard analysis guide and also just sort of a pre-briefing on the 0157:H7.

So, at this time, I would like to turn things over to Dr. Marianne Miliotis who will start us off with Vibrio parahaemolyticus.

INTRODUCTION TO VIBRIO PARAHAEMOLYTICUS RISK ASSESSMENT

DR. MILIOTIS: Good morning, everybody. Today we're going to walk you through the process of our risk assessment as far as we have gotten at this present time. I will start with a brief introduction, an overview of the literature, the time line. I will, once again, remind you

of our objectives and scope that I mentioned in May. We'll go over the questions that the risk assessment has to address.

The best part of the morning is going to be spent on the risk assessment model itself, and I would like to state at this time that we will be focusing primarily on oysters up until--because most of the outbreaks have been associated primarily with oysters and we have very little literature on other than molluscan shellfish.

In our conclusion, we will see are we on target, have we met our milestone, is the risk assessment addressing the questions it was set out to do, what data gaps have we identified, and then we will ask for your help.

Vibrio parahaemolyticus, as you all know, was first isolated in Japan in 1950. It is a gram-negative halophilic organism. That means it likes salty conditions and it is a common inhabitant in marine and estuarine environments, and therefore found within fish, seafood--other seafood-like crustaceans, and molluscan shellfish. It is a important seafood-borne pathogen worldwide.

It causes acute gastroenteritis in humans, and it can cause septicemia. The first time it was implicated in outbreak in the United States was in 1969, in 1971, the first confirmed to be associated with an outbreak in

Maryland. Between 1971 and 1972, there were 13 outbreaks. There were outbreaks in 1982, 1992, and the outbreaks that we are all familiar with in 1997 and 1998. In between the outbreaks, sporadic cases have occurred.

The best characterized virulence factor of Vp is the thermostable direct hemolysin which is a protein that likes best red blood cells, and it is found in over 90 percent of the clinical isolates. Other potential virulence factors which are less characterized but also may confirm pathogenicity to Vp include the thermostable-related hemolysin which is very similar to the hemolysin I mentioned before.

Urease is an enzyme which hydrolizes urea and enterotoxin which produces diarrhea or causes fluid accumulation in animal models, and Shiga-like cytotoxin, which means it destroys intestinal cells. It is very similar to the toxin produced by Shigella, and it will also invade other intestinal cells.

I would like to mention that these factors may occur in combination in the same strain or different factors will occur independently in different strains. For example, on the west coast, you can find TDH-positive, the TRH-positive, and urease-positive strains. We have one isolate from New York which was TDH-negative, TRH-negative, urease-

negative that does produce an enterotoxin. Here is the summary of a literature review that Marianne Ross did on sporadic and outbreak illnesses. The reported cases reflect the patterns of consumption. As you can see, age plays no part in susceptibility. Anyone is susceptible. A majority of white males become ill, because it is a majority of white males that eat raw oysters. People become sick 12 to 96 hours after they have ingested the organism, and the range of oysters ingested ranges from 1 to 109.

Illnesses: Sporadic illnesses can occur all year with a peak between September through October. Outbreaks are more seasonable. The outbreaks in 1997 and 1998 involve over 700 cases. These outbreaks brought many factors and concerns to the forefront. A majority of the cases, as I mentioned earlier, implicated molluscan shellfish, particularly oysters. The oysters came from specific growing area. There was a direct relationship between consumption of raw oysters and illness, thus consumption of raw oysters contaminated with pathogenic Vp has become an emerging microbial food safety concern.

New outbreak strains have emerged, for example, 03:K6 which was found for the first time in the U.S. last year in the Texas outbreak, and we also found, as

I mentioned earlier, hemolysin-negative strains have been isolated from clinical samples.

In the outbreaks last year and the year before, closure of harvest waters was based on illness. Reopening was based on ensuring the public safety, and this took into account two factors: firstly, the change in season and other conditions, particularly temperature known not to be associated with the outbreaks; and secondly, absence of the outbreak strain.

Based on studies conducted, clinical studies conducted, about 25 years ago as well as investigations into outbreaks, FDA indicated that Vp levels should not exceed 10,000 cells per gram. Given the recent outbreaks, fewer cells, probably between 100 and 1000, were required to cause illness. Is the standard still appropriate?

In November 1998, FDA made the decision to conduct a risk assessment on Vibrio parahaemolyticus. What is a risk assessment? It is the process of determining the likelihood that exposure to a hazard such as a food-borne pathogen will result in harm or disease. It helps characterize the nature and size of risks, and the information obtained assists regulators to make decisions on food safety guidance and policies. So the objective of our risk assessment to is provide a scientific framework for the

development of food safety guidelines and policy, to reduce risk of illness caused by consumption of raw molluscan shellfish harboring pathogenic Vp.

The charge of the task force was to evaluate increased risk due to newly emerging outbreak strains, the effectiveness of intervention strategies, the current criteria for closing and reopening shellfish beds and the current FDA guidance of 10,000 cells per gram. The scope of the risk assessment will determine: the relationship between the molluscan shellfish, Vp, and illness; to assess human exposure to Vp based on consumption of raw shellfish; to produce estimates of illness for levels of Vp consumed by different subpopulations; and provide information that can be used in decision making.

What questions will the risk assessment be addressing? The frequently and extent of pathogenic strains in the growing waters, what parameters can predict its presence, the frequency of occurrence in the shellfish, how do levels at consumption compare to initial levels, what is the role of postharvest handling, what intervention strategies can be used.

What do we know about dose-response from epidemiology, clinical, and animal studies? How does the dose-response vary for the different strains of Vp? How

does it vary among humans with different susceptibilities? Is current knowledge adequate? And where should future research be directed to reduce uncertainty in estimating risk?

At this time, I would just like to briefly mention that the FDA is involved in current control plans in conjunction with the ICC. Marleen Wekell and Andy DePaola will talk a little more about that, and if anyone has any questions, please address them to Dr. Bill Watkins.

Okay. Now to move on to the risk assessment model, and we'll give a brief introduction. In order to develop our model, we divided--we had to determine why Vp is hazardous, and this includes the level of pathogenic Vp in seafood at harvest, the effect of postharvest handling and processing, and the ability of the organism to cause illness.

We have determined that it very nicely helped us divide our risk assessment into three modules: the harvest module, which models the relationship between environmental factors and Vp levels at harvest; the postharvest, which we're modelling the growth rate as a function of temperature, effective intervention strategies, and time after harvest to refrigeration; and the public health module, which is further subdivided into

epidemiology, consumption, and dose-response. Here we're modelling the risk of gastroenteritis per eating occasion. It will extrapolate dose-response from clinical trials to sensitive subpopulation and model probability of severe outcomes.

The classical risk assessment components are hazard identification, exposure, dose-response or hazard characterization, and risk characterization. We have identified pathogenic Vp to the hazard. For exposure we, determined the likelihood of intake of Vp, and dose-response or hazard characterization is the relationship of the levels of Vp ingested with the frequency magnitude of illness and risk characterization. We are integrating dose-response and exposure assessments to determine the risk of illness.

Because of the differences in harvesting practices and climate, each module, harvest, postharvest and public health, has been modelled by region and season. The model divided the region into the northeast, which is New York to Maine; the Mid Atlantic, Florida to New Jersey; the Gulf coast; and the west coast. The seasons, we divided winter, January to March; spring, April to June; summer, July to September; and fall, October to December.

On the next three slides, you will see something John Bowers' Math Department decided. It is an

input-output distribution structure of the different models. The outer circles represent the input distributions, which are the factors that influence the output distributions, which are the inner circles. So in this structure, the harvest module, the outer circles are the factors that influence the levels of Vp at the--for the output distribution which is predicted Vp levels in oysters at the time of harvest.

This output distribution of the harvest module in turn acts as one of the input distributions for the postharvest module, and once again these are the factors that affect the output distribution of the postharvest module which are the predicted Vp levels after refrigeration at the time of consumption. So this distribution, the predicted Vp levels after refrigeration, then serves as one of the input distributions for our public health module, and you can see the consumption and dose-response altogether come to combine and give us our predicted Vp levels.

As we go through each module, we will make you aware of certain assumptions that we have made to fill in the data gaps that we have encountered. At the end of our presentations this morning, we will ask you to please consider these assumptions and give us your comments.

Okay. That's it. Thank you. Any questions?

I will now like to introduce Marleen Wekell who will be presenting the harvest module.

PREHARVEST MODULE

CHAIRPERSON WACHSMUTH: As we did yesterday, we'll have a few clarification questions after the presentations, and then as Dr. Miliotis said, we'll do a little discussion following the panels.

It is a pleasure to have Dr. Wekell. She's here from Washington where FDA has a research laboratory. She's ready now.

DR. WEKELL: Thank you. We had several people work on this module: Chuck Kaysner in our group, John Bowers from the Center for Food Safety, Elisa Elliott and Brett Podoski from the Center for Food Safety, and then Atin Datta from DFS. So this represents quite a large group.

This is the organism we're talking about, Vibrio parahaemolyticus, and Dr. Miliotis gave you some information about this organism. In our module, we tried to identify all of the parameters that contributed to the likelihood that shellfish in a growing area contained disease-causing strains of Vibrio parahaemolyticus, and so the very first important thing to keep in mind is that microorganisms are incorporated in shellfish by filter

feeding, and so they reflect the microbiological qualities of the waters in which they are growing.

This is nicely shown by some data from Andy DePaola, where on the Y axis we have the levels of Vibrio parahaemolyticus in oysters, and on the X axis you can see that there is a relationship between the levels in the oysters and amount in the water, and this is true for other organisms as well.

In this case, the levels are little higher. They're 100-fold higher in the oysters than in the water. It is a very important consideration. Our considerations were several: first of all, how it becomes introduced into the environment, into shellfish; how they become established; what we know; what do the data tell us now; and then to try to use those data for predictive models.

First of all, this is an organism that is naturally occurring in the marine environment, and all the conditions, the salinities, other environmental conditions are just right for this organism to survive, unfortunately in the same areas that we like to grow shellfish. It can also be transported in random shellfish growing areas by a number of vectors: animals. It has been isolated from zooplankton, fish, birds, and reptiles.

We have already heard about the virulence factors, but these are very important to keep in mind. These were described some time ago, and I just wanted to point out that the TDH, the thermostable direct hemolysin is another term for it is the Kanagawa phenomenon or KP-positive and we use those synonymously. This is encoded on the chromosome, on the TDH gene, and over 90 percent of the clinical strains are Kanagawa-positive or TDH-positive.

However, in the environment, a much less proportion are virulent, and in 1979, Sakazaki in Japan estimated worldwide that virulent strains constituted something like two percent of the Vibrio parahaemolyticus, and we see now we have some data indicating that in some areas it's much less than that. In some areas in the Pacific northwest, maybe it has three to four percent. That's a little difficult to get a handle on, but I think we have the tools now to do it.

So it's very important that not all Vibrio parahaemolyticus in the environment will cause illness. Only a very small percentage. So you have to not only analyze for Vibrio parahaemolyticus but also determine whether the strains that you are recovering are virulent.

There are some strain differences. I think Dr. Miliotis already mentioned this, that we're seeing some

cases now, and we have been for some years in the Pacific northwest on the west coast, of the presence of the urease enzyme associated with the outbreak strains, and perhaps there is an environmental advantage to the organism having this enzyme urease.

It can break down urea. Perhaps it can utilize the urease environment where other strains cannot. So perhaps it might have an advantage. It does also confirm increased acid tolerance, perhaps help it go through the gut more readily and perhaps even decrease the infectious dose.

Clinical strains on the west coast since 1982 have been mostly TDH-positive or Kanagawa positive and urease-positive. For example, the 1997 and 1998 outbreak strains were TDH-positive and urease-positive. In other areas, the New York and Texas outbreak strains were urease-negative. In the environment, again, we emphasize that they predominantly are KP-negative or TDH-negative, and the urea enzyme can be variable.

Well, what are some of the other ways that Vibrio parahaemolyticus can be transported in the environment? Ship Ballast has been suspected, and certainly this has been known to be a vector for the transport worldwide of organisms, of bacteria, toxic algae from marine toxins. So it's not surprising that this could also be

postulated in some suggestions that this has happened. For example, the 03:K6 strain that was involved in the 1998 outbreak in New York and Texas has been postulated that perhaps this could have been one of the ways that this could have been brought there. It had not been seen there before.

Vibrio cholerae, when the outbreaks occurred in South America, the strain was found in the Gulf of Mexico in 1991 and '92 and reported by McCarthy and Khambaty. Sewage discharge, we always like to blame pollution sometimes for some of our problems, and there have been a number of studies investigating the effect of increased nutrient in the environment, and sewage discharge has been found or other nutrient inputs by a number of investigators to have a direct effect.

The Vibrios attach to chitin. They can digest chitin, and where you have environmental factors that can influence the zooplankton, you can also influence the Vibrio parahaemolyticus.

A direct way of transporting the vibrios in the environment would be relaying them or taking shellfish from areas where Vibrio parahaemolyticus virulent colonies are present and then moving them into areas where they previously were not. So these are all ways that the organisms can be moved around.

We also looked at environmental factors that could aid in the establishment of the organism once it got there, and certainly tidal flushing would be one such factor, although we haven't been able to put this in the model and we need more data to do that, but certainly this could be a factor. For example, in the Pacific northwest in the summertime when we finally do get some sunshine--we haven't had too much this year--we also have low tide, and so quite often our shellfish are exposed to the air with very little water over them so that the sun warms up the water and temperatures can get quite high, and we also see concomitant increases in Vibrio parahaemolyticus at this time.

Predation, we also considered this. We haven't been able to put it in the model yet, but these are all considerations for the future that the Vibrios, as any creature in the environment, also has predators, and Bdellovibrios, phages can also predate.

There's one other factor to keep in mind. Dissolved oxygen maybe also be a factor. It's a facultative organism, and we at this time don't have a way of put that in, and very little data of DO, dissolved oxygen, with levels of the Vibrios.

Well, the most important factor, and many, many investigators have reported on this is temperature controlling the Vibrios. These organisms do very well at higher environmental temperatures, and so temperature is a very key part of our modelling so far, and I'll show you some data that exemplify that.

The organism also is a halophile. It grows very well at moderate salinities, although it can grow full range, and so it does very well in the shellfish growing areas. Particulates and chitin, as we mentioned--I mentioned--can be a factor in the nutrient's indirect effect. So it's not surprising hearing about temperature, that there is a seasonal occurrence of Vibrio parahaemolyticus in the environment, and this has been documented by many, many investigators, Kaneko and Colwell, 1974 reported and many, many others, and this has been found worldwide.

Also, then, there's a seasonal occurrence of the organism in shellfish, and then there is a seasonal occurrence of illnesses, and this can be shown--yes, you can see it. These are data from Tilton and Ryan for the north Pacific, but I think they show quite well what we're talking about. The enclosed circles are water temperature and degrees centigrade, and you can see as you get into the

summer months, July, August, September, the temperature increases.

And then the open circles are the log of the colony forming units of Vibrio species. Those levels also increase in the environment and this is fairly typical data. We see this in the Pacific northwest. It's seen in many parts of the world. So this is a very strong factor that can be used in modelling.

We also have some nuances with temperature. These are data that our group collected some time ago in 1980, '81, of water temperatures in Rocky Bay, Washington, and we can see fluctuations in temperature with the tidal cycle. At high tide, as shown by the red bars, IS temperature. Also this shows the increase with the summer, but the high tide temperatures are a bit low, and the low tide temperatures, not surprising, with low tide the water levels are less and the sun has a better way of warming up the temperature; and these kinds are nuances, at this point we're not able to put into the model, but we're certainly aware of it for certain areas.

The organism partitions in the environment depending on the season, and this shows that factor, I think quite well. This is again Rocky Bay, Washington in '81, '82. It goes with the previous data that I showed, and on

the Y axis we have a log of the organism per 100 gram of substrain and then the month and the year. In the wintertime, usually about the only place you can find the organism is in the sediment. It over-winters in the sediment. It's very difficult to find it in the water or oysters, and it's usually only in the warmer summer months that you'll start seeing it in the water column and in the oysters. The water is shown by blue and the oysters by the red, and I think this shows this quite well. So this helps to show that if you're looking for it in the environment, where it is at what time.

Now, also mentioned, there is a seasonality with the cases of illnesses. These are data from the Washington State Department of Health, and this shows, I think, that quite well, that in the warmer summer months, July, August, September, these are the times when we see the outbreaks, the illnesses with Vibrios, with Vibrio parahaemolyticus, and in the past we saw--most of the illnesses that we saw in Washington State were sports harvest, and really the 1997 and 1998 outbreaks were the first time that we saw such a large outbreak with commercial shellfish.

I also pointed out that some of these years, '93, '97, and '98, were El Nino years. We also blame a lot

of things on El Nino, and this is a time when water temperatures were higher, and we thought this was going to be a really great handle for us, and then when John Bowers looked at temperature data from Washington State, the increased temperatures in the areas where the temperatures were measured didn't quite show that. But I think this shows quite well the seasonality of the illnesses, and this is typical of what is seen in other parts of the country.

Many environmental studies done with the organism, the literature, and the data here is that usually--not always, but usually it's present in the water column when temperatures reach about 15 degrees centigrade--sometimes a little bit less, sometimes a little bit more--but this is usually when we start seeing it in the Pacific northwest. Before that time, as I mentioned, it is in over-wintering in the sediment.

The favorable salinity range for the organism is 10 to 25 parts per thousand, but it can be found below that and above that. Salinity is another factor that we think is quite important, but at this time is very difficult to put into a model, and I'll show you one of our graphs that points that out.

It over-winters in the sediment layer. It also has been postulated to undergo a viable but non-

culturable phase. This would be a dormant phase when conditions are pretty rough for it, and so it can go into this dormant phase when you can't recover it, but it's still alive. This is very controversial. Not all agree that this can happen. We have seen data or evidence in our own laboratory that something like this does happen with this and several other organisms too, but this is a factor to keep in mind, and its relationship to planktonic species is very important for environmental work.

Now, after the outbreak in 1997 on the west coast that Dr. Miliotis mentioned briefly, the Washington State Department of Health started the monitoring, a very extensive monitoring system, and then FDA, our lab in Seattle, also helped out. They had so many samples from Dauphin Island and so many samples to analyze, and these are some of the data that I'll be showing you that we found.

Unfortunately, when outbreaks occur, you can't always get in there as close to the time as you like, and usually the shellfish have been eaten so you can't analyze them. It's very frustrating for us, and so our analytical work really got started a little bit after the outbreak, as soon as we heard about it, and after that time, we found that the total Vibrio parahaemolyticus ranged from 15 MPN per gram to 46,000 MPN per gram.

What has been a concern is, I think, that 10,000 level of concern. These levels were considerably below that, and it was somewhat surprising and of great concern. Of all the samples that we did in FDA, TDH-positive strains were found in 4 of 29 samples, and a sample--these are samples of oysters. They're composites. There's 10 to 12 oysters per sample. So it isn't exactly how many per oyster which is really what we would like to find.

The Kanagawa-positive or TDH-positive strains, the levels of these organisms were three--ranged from 3 to 7.3 MPN per gram. Two were from Washington State. Two were from Oregon. That's very, very low. This also was somewhat surprising to us. Of these, the serogroups were 01, 04, and 05, and all were urease-positive as were the strains, the clinical strains from the patients.

3.2 percent of the Vibrio parahaemolyticus isolates from those oyster samples tested after the outbreak--and we tested something like 300 isolates. 3.2 percent were TDH-positive.

The State of Washington has continued the monitoring program. We just got some data this week from them, and they've been very gracious in sharing their data

with us, and we were very grateful to them and thanked them for that.

In the monitoring that have been done, they take water temperature salinity, Vibrio levels. They do not do--they will be in the future, but in the past they haven't be looking for the virulence, and we hope to help them out, and we've given them some training to do that; but during this time, the water temperature ranged--of course in the winter it's low, only 28 degrees centigrade, salinity 8 to 32 parts per thousand, although our salinities usually are typically around 30 parts per thousand.

They're very high in the northwest, and the levels range from .4 to 110,000 MPN per gram. That was just really a low sample. Eighteen samples were above--only 18 were above 1,000 MPN per gram. In 39 samples, none were detected at all, which our detection level is .3 MPN per gram. And, again, we saw the seasonality, higher levels in the summer, lower in the winter, and so for that reason, not too much sampling was done in the winter.

The Galveston Bay outbreak in Texas in 1998, we also did some work on that and so did Andy DePaola at Dauphin Island. After that point, 30 were sampled after closure from July to September. Total counts of Vibrio parahaemolyticus and TDH strains were also done. FDA has

now developed a probe for TDH. We also have one for TRH, and the for the first time now we can get a better handle on the virulent strains where before it was very difficult to get that, and this will be a powerful tool for us.

Water temperatures in Texas, much higher, 28 to 32 degrees centigrade, and total counts, again below that 10,000 per gram level of concern. We're 40 to 4,300 units per gram. Ninety samples that we ran close to the time of the outbreak, although not as close as we would like to have it, only one of those was a TDH-positive strain, and at that level, it was confirmed that 10 units per gram. So very low, and it was in Serogroup 08 which was not the strain involved in 03:K6. We weren't able to recover that.

We have some indications that some areas may have more favorable conditions than others for Vibrio parahaemolyticus and especially for the virulent strains. We would like to get a better handle on that, and hopefully now with the probe, we'll be able to do that. For example, in 1998--and also 1997, but in 1998, the outbreak, shellfish were harvested from Hood Canal were responsible for 67 percent of the illnesses; and also during 1997. I just got data before I left on Wednesday that we now have 19 cases, case confirmed, in Washington State of Vibrio

parahaemolyticus this summer. Certainly not as much as before. I think our monitoring is working.

And of those, I believe 13 were from the same area, again, and so three years in a row we're seeing this one area which is of great concern. It's also a beautiful part of the state, the canal. Other factors to keep if mind: Are strain differences playing a role here in the northwest? For example, we see the urease-positive strains of great interest and concern to us. One other factor: We haven't mentioned this too much, and I'll mention this now, that we haven't paid too much attention until recently to the oyster itself, the immune function of the oyster, the physiology of the oyster.

The oyster also has the capability of combatting these organisms with hemocytes. Combatting these organisms with hemocytes, and there are recent data, Genthner et al., for instance, published this year showing that there's a seasonality of the ability of the hemocytes to fight and to kill Vibrio parahaemolyticus because other organisms will do much better in the summertime than in the winter. It also depends whether there is a capsule on the Vibrio or not, and then just this month, a paper published--Marianne is one of the authors--looking at immune function of the shellfish, finding that when they were infected with

a certain bacteria, that they weren't as able to combat the Vibrio species. So these are also factors that we really should remember.

There is some indication that the virulence capability on the pathogenicity islands is on the chromosome of Vibrio parahaemolyticus, and perhaps that this can be transmitted, and perhaps this can be transmitted to other organisms and to other strains. For example, TRH, TDH, and urease are very close together on the chromosome, and the GC ratios are lower in this area than they are in the rest of the genetic DNA, and so this is some indication published by Iida, et al. in 1998 that this may have been acquired by the organism. If that's the case, then it can transmit to other organisms, and we are going to be doing some work next year on that.

Well, let's get into the modelling. For the harvest module, temperature certainly is a very important factor. You can't read this, unfortunately, on your handout because I had to use white letters, and of course they're not going to show up when it gets printed. These are data crunched really by Dr. John Bowers in FDA and represent a tremendous amount of data, years and years of data from the north Atlantic, mid Atlantic and Gulf, and these are all from buoy data.

The west coast is from our shellfish specialist in State of Washington, and these are years and years of data. These are temperatures in degrees centigrade. This also shows the seasonality in all these areas, that they're higher in the summer than in winter and also some local areas, the Gulf for example, the temperatures are generally higher than in other parts, and so these are considerations in our model.

A lot of statistics here. Of course you can't read that and that's not what this is for, just to show you that there are--the means were calculated, and this is just one block here for the west coast for winter, and the considerations that were given the mean is the average just across the years, the mean daily water temperature in degrees centigrade, and then the mean of the--an average standard deviation of the daily water temperature, the variance of the daily water temperature, and the variance of the standard deviation. So these are all factors that were calculated, and I think John will be talking about that more.

Now, Andy had published--DePaola--some data that we used here. Vibrio parahaemolyticus in the water column, the water temperature shown in degree centigrade on the X axis and the log of the Vibrio parahaemolyticus per--I

think per gram on the Y. And, certainly, we'd like to see a little bit tighter data, but there usually always are outlyers when you're working in the environment, but there is a relationship, and a linear regression equation is made from these data; and then the salinity, many people say this looks like a scattergram, but this we need to get a better handle on. We don't really have that now.

We all know salinity is a factor, but there the are a lot of other things going on here, obviously, but there is an optimum salinity for Vibrio parahaemolyticus and growth is not as optimal at the lower and higher ends of salinity, and so this may be a factor. Say rain fall when you have salinities going down, but this is a little bit harder one to get a handle on. So for predictive models, temperature is a very, very important factor, and that has affected, of course, by weather patterns, weather perturbations, and the El Nino, La Nina patterns.

Tidal flushing, I think you saw our data showing that tidal flushing can affect the temperature. Salinity, we would like to use that, and we have a diminished salinity in the equations. Zooplankton and dissolved oxygen are factors. We don't have them in our risk assessment right now, our modelling. And these are the

equations drawn from the data, and hopefully John will talk about them more.

Linear regression equations predict, log the Vibrio parahaemolyticus per amount of water and per gram of oysters, and so what we did was just took values, plugged them in and got these which can be shown by three dimensional graphs for the log of Vibrio parahaemolyticus per mil versus centigrade temperature. This is for the water, and then we did the same thing for the concentration of oysters, and then to make it a little bit easier, we just took three different salinities and plugged them into the equation for water, and you can see these are just the estimated means, of predicted means, and then we have the same thing for--

I want to get to oyster. Okay. Here's the oyster. We looked at the values that were predicted and then looked at some of our data and some of that of others, and it's fairly close. For example, in the summer months when the temperatures are 20 degrees centigrade, salinities may be around 30--between 20 and 30 parts we are thousand. We do see means of Vibrio parahaemolyticus around 73 MPN per gram, and this is within the predicted range here.

So to summarize, historical data, most of the older papers that we looked at, virulence was not

determined, and this makes it very difficult to use some of the older papers. Where methods were used the virulent strains were underestimated or missed entirely, and the older methods--and we're still using them--are enrichments, enriching in--and we've seen some of the work that Chuck Kaysner did quite recently. He found that by doing this, quite often the virulent strains--non-virulent strains would outgrow the virulent strains, and you wouldn't even see them sometimes. And so just relying on enrichment method, we have missed this. Now that we have the probe, it will be much, much easier to detect these. Of studies designated or designed to defect virulence, levels of virulent streams were far, far less than the non-virulent, and we already showed--I showed some of that data, two percent, or even lower. Estimates of isolates, of our isolates in the northwest, TDH-positive from the 1997 outbreak, 3.2 percent of them were TDH-positive, and I showed that. It's been estimated maybe throughout the U.S. the level maybe .4 percent. Some areas may be below .4. So it does tend to vary, and this is something that we'll have to watch.

So the virulence factors are very important. One of the key questions--now those modelling equations and the predictive graphs that I showed were for total Vibrio

parahaemolyticus, and so we're really interested in the virulent strains, and so what we have to do in that is then plug in for virulence to look at how many of those are virulent, and one key factor that we kept debating, and it kept coming up, is that do virulent strains respond to environmental factors in the same way as non-virulent strains. We really don't know that. At this point we have to assume that they will grow proportionately to the non-virulent strains in the same way, but we really don't know that, and that is an area that I think we would certainly like to see more really good work done.

Current efforts, I think Dr. Miliotis mentioned this, that the FDA, ISSC has a contingency plan. Dr. Watkins is here, and he can address this more ready, but if a TDH-positive strain is found, the area is closed and can only be reopened with two consecutive samplings of finding TDH-negative strains, and he has all of the bits and pieces of that plan. At the present time, there is an FDA ISSC oyster harvest study ongoing, and we're doing some work on that, as well as Andy and many, many labs are contributing to this effort.

We also have been engaged in training. We have been training states, even universities, and we've trained some of the people from Canada in the use of the TDH

probe, the oxygen and alkaline probes as well as the TRH probe, and so now it's quite exciting. We will have the states able to tell us the percent and the virulent strains where before we did not have that.

So concluding, very clear that seasonal trends are there with Vibrio parahaemolyticus even with the various methods that have been used over the years, but the organisms are higher in the summer. The illnesses are higher, and the shellfish levels are higher.

Geographical areas may be prone to virulent strains. We see that in the west coast in Washington State. There is a suggested high attack rate for some of these strains. That's another crucial thing for our model, that at this point we have to assume that the attack rate for all of the virulent strains is the same, but it may not be. But we certainly need to identify virulent strains, have methods where these virulent strains can be detected in the environment, and we now have that tool with the probes.

So that concludes.

DR. O'BRIEN: I'd like to congratulate you on the clarity of your presentation and the presentation that went right before yours.

DR. WEKELL: Thank you.

DR. O'BRIEN: I have a question, and I'm not sure that my facts are right at all. I have a recollection that there was a publication within the last year showing that at least one strain of Vibrio parahaemolyticus had two chromosomes just like Vibrio cholerae. Are you aware of that finding? And if so, are there, for example, more than one copy of some of the virulence determinants in certain strains compared to others? Do we know that? I'm pretty sure of the observation.

David, help me out. Is that right?

DR. DePAOLA: You're right about Vibrio cholerae, but I'm not--

DR. O'BRIEN: I thought it was a microbe paper by Jim Capers, who is Japanese. Somebody is going to have to help me out on this--that Vibrio parahaemolyticus also had two copies like the Vibrio cholerae. It wouldn't be surprising if Vibrio cholerae does.

DR. DePAOLA: Yes. Andy DePaola. You're correct. There was a paper, and there are two chromosomes on Vibrio parahaemolyticus, and there's a copy of the TDH gene on both chromosomes, however the one copy has--it's much more functional on that copy because of the promoter, and that's responsible for virulence more than the copy on the other gene.

DR. O'BRIEN: In that strain, that was the case. One was--the levels--there was actually better transcription with the copy on one--

DR. DePAOLA: That's correct.

DR. O'BRIEN: --chromosomal copy than the other. I just wondered, though, if it might be important to take a look at some of the strains that you're considering virulent to see whether this is a phenomenon that's across Vibrio parahaemolyticus, whether it was just an oddity. I suspect it's across Vibrio parahaemolyticus, and the fact that in one case one copy wasn't transcribed well in the lab may or may not be true unless there's a lot of commonality in Vibrio parahaemolyticus with other isolates of Vp.

DR. DePAOLA: Yes, I think that merits further investigation.

CHAIRPERSON WACHSMUTH: Can the chair ask a question of Alison and/or Andy? If transcription is a problem, then using the probe to detect the gene could be misleading; is this true?

DR. WEKELL: No, our probe detects its presence on the chromosome.

CHAIRPERSON WACHSMUTH: But if it's not transcribed--

DR. WEKELL: Oh, I'm sorry.

CHAIRPERSON WACHSMUTH: --then the gene could be there, and you could still have a--

DR. WEKELL: I hope not.

DR. O'BRIEN: If there are two copies of the gene, and one is transcribed poorly, and the other one is not, and they are on the same restriction fragment--which I don't know--first of all, you might not be able to tell there are two copies, which as you know what's nearby and what size restriction fragments you've got.

But you're right, if transcription is poor or good, all you're getting is whether the gene is there. You're not getting the expression in surveying. I'm not in any way trying to take away from the idea of surveying. I'm just answering your question.

DR. WEKELL: No, it's very interesting.

DR. DePAOLA: I think you bring up a very good point, and what we'll have to do is take these isolates and do further subtyping, and in fact we're working on an interagency agreement with CDC to have them conduct subtyping tests, and we hope to also be working with the group of FDA in D.C. to do some animal testing on these isolates to confirm their pathogenicity and the effectiveness of the probes.

CHAIRPERSON WACHSMUTH: Thanks. Bob?

DR. BUCHANAN: Can I just make a general comment because our time is very limited? The focus of today's session is to evaluate the current risk assessment, and while the identification of future research is obviously part of that in terms of the data gaps that we face, if we can focus more on whether or not this has any direct effect on their interpretation in modelling would be much more effective as we go through this discussion.

CHAIRPERSON WACHSMUTH: I think this is very relevant.

Dr. O'Brien?

DR. O'BRIEN: Well, I think it is relevant if what they're looking at for virulence determinants are not expressed and are not relevant. So I think it is an important point if they're correlating virulent strains with whether or not they're going to cause an outbreak.

DR. BUCHANAN: Do you have a suggestion on how that should or could be incorporated in the model?

DR. O'BRIEN: No, but I was actually asking a question of clarification of virulence determinants. This point had not been raised, and I was just trying to remember my facts to see if it played a part.

DR. BUCHANAN: What I'm concerned with is the way the discussion was going. It was a very good discussion

that was starting to take place about the basic biogenetics of Vibrio, but our time frame here this morning, they have a very--

DR. O'BRIEN: Fine.

DR. BUCHANAN: No. These things should be identified as future research areas that this identifies and passed on, but a long-term discussion on it is going to take away from the time of questions that we really need to focus on in terms of answering their questions.

CHAIRPERSON WACHSMUTH: I appreciate your assistance to the chair, Dr. Buchanan.

David?

DR. ACHESON: Well, I'm not sure I dare ask another question, but we heard in the first presentation that TDH was associated with 90 percent of the strains that I believe were isolated from clinical illness. How significant do you believe that other 10 percent is? I mean, obviously you're basing all your virulence characterization, PCR, on TDR. Is that 10 percent a big deal?

DR. WEKELL: They're actually usually around 97, 98 percent of the clinical strains, and the TDH-negative, quite often they are TRH-positive, and that hemolysin does not affect red cells as effectively as the

TDH so that we get a very weak chemical reaction, and we do have a probe for TRH, but in the literature quite often those strains are TRH-positive. I think there's been one that was TRH and TDH-negative, but that's very rare. I hope it stays that way.

CHAIRPERSON WACHSMUTH: Okay. Stephanie.

DR. DOORES: This might be a rather unusual question, but on the east coast the normal consumption pattern for oysters is from September to April, kind of those months that end in R or have a R in them. Is that kind of short-handed season, so to speak, used also for the west coast, or do you normally eat oysters year round?

DR. WEKELL: Oysters are consumed year round on the west coast.

DR. DOORES: Okay. Because we tend to have--I mean we can eat them year round here, but we tend to have that shortened season.

Also, are plankton levels measured in the--on a regular basis there to see how they correlate with Vibrio parahaemolyticus?

DR. WEKELL: Not too much. In research studies they've been shown to correlate, but for monitoring purposes, usually they're not done, but we were aware of this, and I think it's something we need to keep in mind

when we start modelling, but those data generally aren't available for typical monitoring purposes.

DR. DOORES: And the last question I have is would these be areas in which people would swim, and do you see any Vibrio parahaemolyticus occurring in these people from swimming in these areas?

DR. WEKELL: That's a good question. I don't think we have, and it would be possible, although keep in mind that the water levels are a hundredth of the level of the oyster, and so in the water column, the levels may be below infectious dose.

DR. DOORES: But I was just wondering if the people that became ill that were not associated with oysters might be that sort of background noise but they really had been swimming in the area.

DR. WEKELL: It's possible. We've certainly seen that we E.coli 0157, but usually the levels in the water are fairly low, in the 10 to 70 or less Vp per gram.

CHAIRPERSON WACHSMUTH: Mike?

DR. JAHNCKE: Mike Jahncke, Virginia Tech. I have a question. That particular day in Washington that was associated with one of the outbreaks, is there--so far, is there anything particularly unusual about that, or is it

simply perhaps just lack of flushing or circulation patterns or something?

DR. WEKELL: Yes. We think that the lack of flushing may play some role because Hood Canal is kind of an inland salt water body, and flushing is not as great there as in some of areas, and you can also get some warming, water warming in that area. We haven't had the resources to study that in any great depth, and that would certainly be a very good area for environmental study.

There is a study going on now at the University of Washington by Dr. Russ Herwick, and we've been sharing data with him. So hopefully we can get some answers on that, but we need oceanographers involved in some of these studies.

DR. JAHNCKE: Are there any particular harvest sites within that particular day that have, you know, a higher prevalence?

DR. WEKELL: There are, yes. Yes.

CHAIRPERSON WACHSMUTH: Okay. Time is running short. Bob, as quickly as you can and then Mel and then we need to end.

DR. BUCHANAN: Marleen, what were the confidence intervals around your regression lines?

DR. WEKELL: I think Dr. Bowers can answer that, but they were pretty big.

DR. BUCHANAN: Do you have a rough ballpark? One, two log cycles?

DR. WEKELL: It's still pretty rough.

DR. BOWERS: John Bowers. We didn't calculate the confidence intervals. We were just looking--we just calculated the mean and the variance throughout the regression as an estimate of population various of the Vibrio levels.

DR. BUCHANAN: Well, I guess the question is there really much significant difference between those different lines? You did the three different salinities. There didn't appear to be much difference based on salinity.

DR. WEKELL: There may not be.

DR. BOWERS: I'll go into that in my time.

DR. BUCHANAN: Okay.

DR. WEKELL: Salinity is really hard to get a handle on.

CHAIRPERSON WACHSMUTH: Okay. Mel, and then I need to move on.

DR. EKLUND: Mel Eklund. I have a couple of questions for Marleen. One thing that's bothered me a little bit from the standpoint of yesterday with Listeria

and also today with Vibrio parahaemolyticus is the tremendous range we get as far as the number of organisms during an outbreak. Part of this is methodology, I'm sure. The other is maybe not having the same sample representing for the person who consumed it.

As far as methodology is concerned, I'm glad to see you're using the probe now because I've always been quite concerned during enrichments for an NPM system. I think you have during the summertime, during the height of Vibrio growth, you can have as much as ten to the ninth phages for Vibrio parahaemolyticus. When you do the enrichment technique, you can then find a negative tube. Was the negative during the night?

So I think this is something, that in the whole risk assessment from yesterday and today, we have a concern of the number of organisms that have needed to cause an illness.

DR. WEKELL: We've been concerned about phages and alkaline. So I appreciate your comments.

CHAIRPERSON WACHSMUTH: Okay. Thank you. Thank you, Marleen.

POSTHARVEST MODULE

CHAIRPERSON WACHSMUTH: Our next presenter is Andy DePaola from the Dauphin Island laboratory.

cc

DR. DePAOLA: Good morning. I'm real happy to be here.

This morning's presentation on the postharvest module will identify the data sources and try to explain the rationale and assumptions used to model the effects of postharvest practices and potential mitigation on the levels of Vibrio parahaemolyticus in shellfish from harvest to the point of consumption.

I have had quite a bit of help with this presentation. I would especially like to thank Dr. David Cook from our laboratory and John Bowers from the Division of Math.

This is a slide I showed back in the meeting in May, and I thought I would just review some of the oyster processing that occurs at harvest. This is done primarily with dredges and hand tongs on the Gulf and Atlantic coast, whereas in the Pacific coast, the oysters are quite often collected at low tide in intertidal areas, and the harvesters will pick them up and place them in baskets, and then when the tide rises, they'll come back and retrieve the oysters.

They are culled after they're harvested, and this is a process to remove small shellfish and shells that maybe attached to the market oysters, and then they're

stored aboard the harvest vessels, and in most cases these are small vessels, and they lack mechanical refrigeration.

The oysters are usually held in the shade in baskets or burlap bags until they're landed. They are transported to the processing plant, and the transport may or may not be with the refrigeration. If they go across state lines, refrigeration is required, and some states may require refrigeration.

When they arrive at the processing plant, they're usually placed under refrigeration, or it's mandated they're placed refrigeration from 45 to 50 degrees Fahrenheit which is about seven to ten centigrade.

There's two processes. On the left here we have processing for shell stock, and this is in-shell, live whole oysters, and this is a very simple procedure. The mud and debris are washed from the shells, and then they're re-boxed, usually in cardboard boxes, and then returned to refrigeration from the wholesaler to the restaurant all the way to consumption, usually between seven to ten degrees centigrade.

On the right side, is the process used to obtain oyster meats, and the oyster meats are removed from the shell by severing the abductor muscle and putting the whole animal into metal or plastic containers submerged in

ice. Afterwards, the shell fragments are washed from the meat in a process called blowing. Then they are packaged and stored on ice to the point of consumption. Usually this is the process that is used for cooked shellfish, but occasionally these will be consumed in shooters.

So really the question we're trying to get out today is will the shellfish industry practices affect the Vibrio parahaemolyticus risk, and are there mitigation strategies that would reduce the risk of Vibrio parahaemolyticus. This slide, we go from risk to densities, and what we're assuming here is that there is some relationship between density and risk, and we'll leave that up to Don Burr, the Public Health Section to elucidate this relationship.

So what we're looking at is do industry practices affect these densities, and our approach is to look at the levels at harvest to the point of consumption, and when we say industry, we're talking all the way from the harvester to the server at the restaurant. Three factors affect the levels of Vibrio parahaemolyticus at the point of consumption, and these would be the major points of the talk. The first would be the level of Vibrio parahaemolyticus at harvest. The second is the growth

before the oysters or other shellfish are refrigerated, and, finally, die-off during refrigeration.

One basic assumption that's going to go through this module is that all strains of Vibrio parahaemolyticus grow and survive similarly, and there's no difference in the way that pathogenic and non-pathogenic strains behave, and this is primarily due to a lack of or a very small amount of data, but the small amount of data that's there does support this.

Once again, this module will be divided in seasons and regions, much as Marleen had described, and she's already gone through the seasons and the regions. Okay. So what we'll begin with is the levels of Vibrio parahaemolyticus at harvest, and this is the study that she cited, and these are just summaries, and what we see is that the highest counts were along the Gulf of Mexico, and the low counts on the Atlantic and Pacific. This is for year round data, but this gives you an idea of the effects of region.

And similarly--this is the same study--the effects of season. We have our highest counts in the spring and summer, and, once again, the lowest in the winter, and this is the mean of all regions, Pacific, Gulf Stream, and Atlantic come up to these values, and this is the study that

would be used to model the levels at harvest, and hopefully we'll have the simulations available on the next step for the various regions and seasons.

Okay. Now let's move into the growth before refrigeration. We've considered three factors important in this, the time to refrigeration between when the oysters are harvested and first placed under refrigeration, the surrounding ambient air temperature, and the growth rate.

The first factor, time to refrigeration, we have two sources of information. The FDA dealer survey for the effectiveness for the interim control plan which was used to--or implemented to reduce the levels of Vibrio parahaemolyticus in oysters and a memorandum for the Pacific Coast Shellfish Growers Association in the dealer study that was conducted in 1995 and 1996, and the measures actually first came into effect in 1996. So the '95 data represents before the interim control plan, and the '96 after it was in effect. Three states, Florida, Louisiana, and Texas, were sampled each week during the months of September and October for both '95 and '96. The Pacific Coast Shellfish Growers Association, in their memo, they stated that the majority of the oysters harvested on the Pacific coast were from intertidal zones and they were harvested on low tide and refrigerated within four hours, and they stated the lack

of refrigeration time was due to the short tidal cycles and the close proximity of processing plants.

We have a number of assumptions that we're going to use to extrapolate the small amount of information to other regions and seasons. First of all, the 1996 data from the study is representative of current practices on the Gulf coast during the summer. The 1995 data, which was before the ICP, is typical of the Gulf coast during the winter when the ICP requirements are less stringent and also representative of the Atlantic coast year round, and the Pacific coast information is representative for the Pacific oysters year round.

Other assumptions are that oysters are harvested at a constant rate throughout the day. In other words, those that are harvested in the beginning of the day, say between six and seven o'clock, there would be a similar amount harvested in each hour after that until the end of the harvest day at which point you would take about one hour for the vessel to return to shore and place the oysters under refrigeration.

And, finally, that this distribution follows a Beta-Pert, and I'll show a slide on this, and if you have any questions, John will be happy to answer those.

Okay. So these are the estimates that we're using, and the Gulf states are divided up between what we'll call the summer, April to October, and the winter, which is November to March. They're further divided between Louisiana and other states, and the reason for this is that the shellfish growing areas in Louisiana tend to be a lot more remote than what's found in other states, so it takes a little bit longer to get the oysters back to refrigeration. And in the summertime, this takes about nine hours in Louisiana and about six to seven hours in other states, and this is based on the '96 data, and the '95 data is a couple of hours longer for Louisiana and about an hour longer for the other states.

Okay. And the north and Mid-Atlantic, we're assuming is based on the data from Texas. We feel it's going to be about 7.7 hours. In the Pacific, it would be the four hours stated in the memo.

Just for your information, this is what a Beta curve sort of looks like. It's skewed over here on this end. This is the length of the harvest day for the Atlantic coast, and the typical time is about eight to nine hours, and what you see is that there is more of--more likely that the times are going to be between about six and ten than it is for three to four.

Now, don't confuse this to the average time that an oyster is refrigerated. This is the mean maximum times. The oysters that are harvested at the first of the day, the time to refrigeration will be a different distribution, and that's assuming the constant harvest throughout the day and the one hour of transport time. So that covers the time to refrigeration.

The next factor is the surrounding air temperature that affects Vibrio parahaemolyticus growth, and these are shallow water buoys that are representative of each of the regions in which we used to collect the ambient air temperature. Marleen used the same buoys to collect ambient water temperature.

There are also a number of assumptions here, and that is that the air temperatures of the shallow water buoys are in fact predictive of the postharvest oyster temperature. We've used a noontime value since this is somewhere in the midpoint of the harvest day, and we've assumed that the oyster temperature changes to the area temperature within one hour of harvest, and this distribution is a truncated normal distribution. Once again, John will speak more about that.

Okay. These are the values that we obtain through the buoys, and what we see is for the Gulf coast is

that we have warm temperatures throughout the spring and summer, whereas there is a much narrower period of time in the North Atlantic and Mid-Atlantic, and in the Pacific we have our coolest temperatures.

Now we move to the next step beyond time to refrigeration and air temperature, and this would be the growth model, and the source here is Gooch, et al. She's prepared a manuscript. She's with the National Ocean Service Lab and corroborated with us in this study where natural Vibrio parahaemolyticus in oysters was determined at 12 times the monthly intervals.

The design was to harvest the oysters and place them at 26 degrees and take samples at 0, 5, 10, and 24 hours and then transfer the remaining oysters to three degrees and sample them after about a two week period. Only one temperature was used in this study, 26 degrees, which is very typical of the Gulf coast during most of the period of April through October.

So we had to go to the second study, Miles, et al. He did not use--they did not use oysters. They used the model broth system and varied temperature and water activity. So we're using that study to get values for temperatures other than 26.

On the oyster data, this is a curve that was calculated for the growth of total Vibrio parahaemolyticus numbers in oysters. You see a very brief lag period, and exponential growth starts to level off about 24 hours and reaches a maximum of around 100,000 per gram. Just to summarize what this curve says, is that the lag time is about one hour, shortly less than the one-and-a-half-hour doubling time and a maximum growth of a little over three logs.

These are the assumptions that we're using on the growth curve, and the first assumption is that the growth rate on the broth model system is about four times greater than oysters, and this is based upon the observation of 26 degrees in the model broth system and the fact Vibrio parahaemolyticus did multiply four times faster than it did in oysters, and this is probably because of it was a pure culture and didn't have other factors such as phages and other things that might be affecting its growth, and we're assuming that this relationship of four times is occurring throughout the growth range, and the growth range is assumed to be 10 degrees, which is the temperature below which most strains of Vibrio parahaemolyticus won't grow and 35 degrees, which is probably at or above the maximum temperatures that oysters would ever be stored.

We'll also assume that the lag time will be negligible, and that's because the oysters are taken out of waters that the temperature is usually the same that it is in the surrounding area, and that the maximum growth is not affected by temperature. In other words, if stored long enough at the lower temperature, they'll reach the same density but a longer time than they would at the higher temperature.

And, finally, we're assuming that salinity does not affect the growth, and the data suggests that the salinity in the oysters is about the same as the optimum growth for Vibrio parahaemolyticus. These are some simulated curves. The one in the middle here is the 26 degree which is not simulated. This is what we observed in the investigation, and then we look up here at 32 degrees and also at 20 degrees, and looking here at 10 hours, we have about one and a half log growth at 26, about two and a half log growth at 32, and less than a one log growth at 20 degrees, and the simulation will take all the possible temperatures and factor this in for Vibrio parahaemolyticus growth.

The final part of the Vibrio parahaemolyticus levels at consumption is the die-off that occurs during refrigeration. We have three factors: the cooling time

after oysters are placed under refrigeration, the length of time they are refrigerated, and the die-off rate during refrigerated storage.

And the cooling rate, one of the things you have to keep in mind is that the oysters are placed under their warm temperature, and in fact die-off is not occurring. That growth is actually occurring until oyster temperatures come down to 10 degrees, and the rate of this cooling is dependant on the efficiency of the cooler, the quantity of oysters placed in the cooler, the temperature of the oyster, and how they're arranged, whether they're stacked so air flow is possible around the sacks or containers of oysters, and unfortunately there are no authoritative data on these factors. So we created a little bit of data from some preliminary experiences, and we looked at probably the best case scenario which is a single oyster which we inserted a temperature probe into its tissues. We brought it out of the water in 30 degrees and placed it into a three degree cooler, and what we observed is that the temperature would drop about a half of degrees centigrade per minute. However, in another study where we took 24 oysters and put them in a Rubbermaid container, it took seven hours for them to cool from about 26 degrees to 3

degrees. So these experiences show that the cooling rate can be dramatically affected even in the same cooler.

So we're assuming that there's quite a bit of variation in industry practices, not just from an environmental standpoint of how warm the oysters are when they're placed in, but certainty the efficiency of the coolers and other factors, and not only is there a variation of these practices, there is uncertainty because of the lack of data, and because of this, a rectangular distribution with a minimum of about 5.5 hours, or half an hour to a maximum of about 10 to 12 hours, would be required for them to cool to 10 degrees.

Fortunately, for the length of time of refrigeration prior to consumption, we have much better data, and that was generated through the ISSC/FDA retail study which was completed in June, and this was a nationwide and seasonal survey of oysters at the point of consumption, and when the oysters were collected, the meantime was about 7.7 days after their harvest, and we're assuming that would be about an average time for them to be consumed. A range of about 1 to 28 days was found and this would follow a Beta-PERT distribution. On this point, there would probably be more harvested closer to one day than to 28 days.

For the die-off rate, we're using the same study as we used for the growth, and this was the part of the study where the oysters were transferred from 26 to 3 degrees and then sampled after about two weeks, and the die-off rate was about .003 logs per hour. This is total Vibrio parahaemolyticus. The assumptions are that the die-off is constant from three degrees to ten degrees, commercial coolers are required to be between seven and ten degrees, and that the lower levels examined were the result of die-off, and that is a result of failing to recover the injured cells.

We don't have our model simulations available, but when they become available, we will compare them to the observed values during the retail study which was done throughout the year at the various regions, and what we see here is that for along the Gulf coast, we have values of over 100 per gram through almost three-quarters of the year, and this also occurs in the Mid-Atlantic but over a much shorter period of time, and then on the Pacific coast, there was much less data available here than on the Gulf coast, whereas about 30 to 40 data points for each season. So probably 10 or less and through much of the year wasn't detected, and even during the summertime the values are slightly less than one per gram at the point of consumption,

and whereas it looks like there was growth in the Gulf coast, and maybe the Atlantic oysters. It looks like, on the other hand, there may have been an actual decline in the Pacific oysters, and this is consistent with the cooler temperatures and the shorter times to refrigeration.

We also looked for pathogenic strains in the retail study. Of 137 samples that had been tested at this point, between three and four percent of the samples actually harbored strains. We don't have solid numbers, but they would probably be less than 10 in most of these samples, and overall, slightly over 2,000 isolates or about a .3 percent of the total population was pathogenic.

This is actually a fictitious curve right here, but this would be a normal distribution of what we are assuming to be in the survey, and this would be our simulation. Now you would see that each one of these would have a mean of about one and that the simulation would be a little bit broader because this went over about 10 years of data, whereas the survey was done over a single year. This is the type of distribution that we hope to generate from the simulation, and this is a sort of comparison.

I'm going to move to the next phase which is potential mitigation, and this is the slide that I showed back in May, and I'll just review these. The National

Shellfish Sanitation Program has a time-temperature matrix that was used to control Vibrio parahaemolyticus numbers. Depuration or relaying oysters are transferred from, quote, contaminated to non-contaminated waters. This is usually done for fecal forms and depuration as a controlled situation, whereas relaying is just another area in the environment; and, finally, most of the focus will be on postharvest processing techniques.

The NSSP model, which has undergone some proposed changes since the last meeting, but what's currently under effect is that between April and October in the states where there has been multiple vulnificus cases, the oysters needed to be under--have to be under refrigeration between 10 to 14 hours, and this time is based on water temperature. In other months, it's 36 hours, and in other states the times are much less stringent than on the Gulf. What we've seen from the survey data, the data from the dealer and from whatever information we've been able to get, is that oysters are generally refrigerated much sooner than what the matrix actually specifies as a minimum requirement for the time of refrigeration. So we don't think that this matrix is going to have much effect in reducing what we're currently seeing, that if the times to refrigeration were reduced, it does look like it may have

some effect on reducing levels, at least along the Gulf coast and perhaps along the Mid-Atlantic during the summer.

The depuration of vibrios, there's a problem with this in that there's conflicting data. Early studies in 1980 showed that there was potentially a five-log reduction in laboratory-infected strains and also a reduction to non-detectable levels in naturally contaminated, however some a subsequent study used in naturally-infected oysters showed that there was no difference between or after depuration of Vibrio parahaemolyticus levels, and another study that was done--these two were done with oysters, and a study with hard-shelled clams also showed there was a very small reduction, less than--or approximately one log which agrees with the data here for the naturally-infected oysters. So we're assuming that depuration is not going to be a reliable process for reducing Vibrio parahaemolyticus numbers.

Finally, to the postharvest processing, there are a number of technologies that have been processed, and these include mild heat treatment, freezing and hydrostatic pressure, and irradiation, which you can currently use any of these three. This is not approved for use at this point, and most of these have come about for the control of Vibrio parahaemolyticus to reduce it to non-detectable levels which

are defined as less than three per gram. If you are doing this, you need to have a HAACP plan, and if you're able to achieve this and demonstrate it, you have to be able to-- you're allowed to label your shellfish as processed to reduce Vibrio parahaemolyticus to non-detectable.

The mild heat treatment is the only process at that point that has that label reduced to non-detectable, and this study was based on work by Cook and Ruple. It showed that you could get about a six-log reduction in Vibrio parahaemolyticus levels in shucked oysters if they were heated 50 degrees centigrade for five minutes, and there's preliminary data from our laboratory that shows that Vibrio parahaemolyticus and vulnificus responds somewhat similarly to heat at 50 degrees. Ameripure, in fact, has patented a process like this to reduce Vibrio parahaemolyticus to non-detectable levels.

Another technique that's currently in use is freezing, and the same study by Cook and Ruple showed about a four to five log reduction in natural Vibrio parahaemolyticus in oysters when they were frozen at minus 40 and stored for three weeks, and it will show that Vibrio vulnificus and Vibrio parahaemolyticus respond similarly in frozen shrimp as. Industry is currently marketing these

frozen oysters and have applied for FDA approval for labelling.

A new process that's gaining a lot of popularity is hydrostatic pressure. I didn't present this in May, but in June there was a publication that they didn't show data, but at the end of the study they reported that they could achieve greater than a six-log reduction treating oyster homogenates at 200--one of these is, I think, 10 atmospheres of pressure for about 10 minutes and the process currently used to shuck oysters. When the oysters are treated with this pressure, they open and the abductor muscle separates from the shell, and the industry has requested FDA approval for this labelling.

So, in conclusion, the Vibrio parahaemolyticus densities were higher at the market than at harvest, and this was primarily for the Gulf coast and for some of the Mid-Atlantic. This was not necessarily the case for the Pacific. We know that Vibrio parahaemolyticus can multiply in oysters if they are not refrigerated and that the densities will decline slowly during refrigerated storage anywhere from about a half a log to a log, but you can get a large reduction in Vibrio parahaemolyticus with mild heat treatment, freezing, and we can add hydrostatic pressure to that also.

Thank you.

CHAIRPERSON WACHSMUTH: Thank you, Andy.

Questions? Bob.

DR. BUCHANAN: Point of clarification, Andy. As you went through the different interventions, were your assumptions about their efficacy, assumptions that you're going to start with now, or are these things that you'll be looking at as you go through the evaluation?

DR. DePAOLA: I think they are assumptions that, as I said, they should be as effective on the pathogenic as the non-pathogenic strains, and basically the way we'll look at this is as a simulation. When I showed the one normal curve where the density was log one, this would tend to shift it to the left to where you'd have the same distribution, but it would be--the mean would be around minus five, and you'd have about two logs of variation on either side of that.

DR. BUCHANAN: Okay. I was thinking most of your--about your slide on depuration. Are you making the assumption in your model that depuration isn't effected, or are you asking the committee to determine whether or not which of those studies you should use?

DR. DePAOLA: Well, certainly we'd like to have the committee's input on which of the studies--which of

these processes or studies that we should use, and the experience of--and I know the vulnificus world is something that some people don't like, but when laboratory-infected strains of vulnificus were put into oysters, they could be depurated, but when you look at natural populations, it was found that they are much more difficult to be depurated, and since there was mixed, we would not consider that as a reliable way of reducing Vibrio parahaemolyticus either.

DR. BUCHANAN: So we can assume that these difficult assumptions will come up during the question and answer period?

DR. DePAOLA: I certainly invite comments on our assumptions.

DR. BUCHANAN: Okay. Thank you.

DR. RUPLE: Andy, I just want to have you clarify something. I'm not sure that I understood. When you are calculating your densities at harvest for your model, are you basing that on just the one study that you showed? I would think with the survey data that's available there maybe other studies, and understanding that what you reported was probably with new methodology since it was reported as colony forming units per gram, and there may be some problem with comparing that to MPN data that may have been used in the past.

DR. DePAOLA: The study was actually a direct plating method based on membrane filtration using a hydrophobic grid. It was older data, and it's probably not as good as the new direct plating methods in where colony--a list of all the colonies are identified there. We would pick five and estimate a percentage of 20, 40, 60, or 80 percent that were positive.

It was the study that we're currently using for the harvest module, and the reason that we've selected that study is that it was done year round and reflected, you know, each of the four seasons, and it was done nationwide. So there is data from each of the regions, and there was a constant methodology.

Part of the problem that we have is that some studies use difficult methodologies with varying sensitivities, so it's like comparing apples to oranges when you compare one region to another. Now, certainly as the data comes in from the ISSC state monitoring programs, and we have a number of states in each of these regions, and they're collecting data every week or every two week intervals, that's going to certainly be much stronger, and currently there's--that effort just really got underway in earnest in the last couple of months, and in some states it hasn't gotten underway yet.

So, certainly, we will shift to using that data and also use that data to determine whether our model is in fact predicting what's actually occurring.

DR. GRIMES: Jay Grimes. In this slide and a couple of your previous slides, you have discussed decline as a result of refrigeration. My question is what did you do to distinguish actual die-off from the entry into a dormancy state by the cells?

DR. DePAOLA: I assume you're talking about the viable and non-culturable.

DR. GRIMES: Yes.

DR. DePAOLA: This is okay to do with water where you have a known amount of cells and you can look at them under a microscope and everything. The problem is when you're dealing with oyster tissue, you have a lot of florescent material, and I don't know of any way that you can distinguish viable and non-culturable cells from culturable cells.

Now, we did use several methods, and one of the methods was a method where a repair step or magnesium was added, and filters were plated--and nylon filters were plated with homogenate, and after several hours they were transferred to TCBS, but magnesium is a substance that's been showed to aid in the repair of chill-injured cells, and

in fact this method did not give any different or any higher values than our probe method that did not use magnesium nor the MPN method. They were all about equivalent.

DR. GRIMES: Thank you.

CHAIRPERSON WACHSMUTH: Mel.

DR. EKLUND: Yes. This is Mel Eklund. This is a follow-up question to Bob Buchanan's comment on depuration. You may have said this, and if you had, I apologize. I didn't hear it. The attachments for the depuration in laboratory studies, as I recall from the laboratory, there was some signs of depuration, as I recall, but with the natural there wasn't. So they've lost the attachment ability is the idea behind this for the laboratory cultures?

DR. DePAOLA: Well, natural populations, when you have a laboratory infection, usually a single strain is added, and it's grown in a laboratory medium, and it's not a natural situation whereas in an oyster there is quite a bit diversity. Each strain that's been examined from oysters, when it's been fingerprinted, has been shown to be different than the next strain.

So when you have a diverse population like this, they maybe less susceptible to predation by phages or other things, and we just feel more comfortable using

naturally occurring strains, and fortunately we have the methodology now that we can effectively enumerate naturally occurring strains than to have to go with--and they do occur naturally with other bacteria like Listeria and Salmonella.

It's very hard to find material that has pathogenic strains, and we had the same problem, to a certain degree, with Vibrio parahaemolyticus in that it's hard to find oysters that have naturally occurring TDH-positive strains.

CHAIRPERSON WACHSMUTH: Okay. We have one more question. Oh, two more. Spencer, then Swam.

DR. GARRETT: Thank you. Spencer Garrett with the Natural Marine Fishery Service. Andy, one of the principles of a risk assessment is as new data becomes available, obviously it will be used and the assessment updated. So I took from your comment that as the state data becomes available, then regardless if this assessment is produced in December or January or whatever, that new data will be cranked into the simulation model; is that a fair understanding?

DR. DePAOLA: Yes. I think we will do that as it becomes available, and I think it's been stated that this is an ongoing process, and we certainly look forward to that data. It's just unprecedented to get that kind of

cooperation from the states to provide the data to FDA and also to provide the strains.

MR. SWAMINATHAN: Bala Swaminathan, CDC. Andy, I just wanted some clarification on this. You mentioned that the TDH-negative strains have exactly the same growth characteristics as TDH-positive ones. Dr. Wekell mentioned that this may not be the case. In fact, she gave the example of alkaline water and TDH-negative strains overgrowing the TDH-positive strains. My question is do you have some, you know, disagreement within the FDA about this, that more work needs to be done? Could you clarify that, please?

DR. DePAOLA: I think she is talking about the factors that affect the distribution and the environment, whereas I'm talking about the factors that affect their growth and survival after the oysters are taken out of the environment, and certain alkaline peptone may be much different than what you would find in an oyster, but we do have some data where we have incurred levels--once again laboratory-infected--where we added the 03:K 6 strain that's responsible for the Texas and New York outbreaks to the water, and the oysters accumulated that, and then we stored the oysters at 26 degrees.

We did not sample at regular intervals, but after 24 hours we did have a three-log increase in that strain, and we'll continue along this line of work, and that was some data that I presented back in May, and that's just an assumption at this point.

Basically, we have no data that contradicts this. I'm not saying that that assumption is true, but as the data comes in, obviously we will modify our assumptions.

CHAIRPERSON WACHSMUTH: Okay. We're just a little behind. Thank you, Andy.

CONSUMPTION

CHAIRPERSON WACHSMUTH: Our next presenter is Dr. Michael DiNovi.

DR. DiNOVI: Good morning, everyone. This morning I'm going to present a very brief summary of the data that we're using for the model consumption of oysters. I assure you we will not overrun the time on this particular section.

As you've heard, we're using a 4-by-4 grid of regions and seasons, and as you can see, as we've mentioned earlier, there is a seasonality to growth. These data are mostly from the National Marine Fisheries. You see 1998 here. I have data for the last 10 years that we will use to model variability in landings from year to year.

Briefly, you can see on the bottom the variability and only about a factor of two over these last 10 years. The data on the left was sent to me by the west coast growers and actually is a projection of next year's landings based on 1990 through '95 with a growth rate assumption built in. These data will be used, as you can see, in conjunction with the next few slides to allow the model to determine whether or not--depending on what you want, the number of meals, the size of meals, or how much Vibrio will be consumed in a given meal.

All right. The assumptions that we're using here are approximately 40 to 50 percent of oysters are consumed raw. I have data from a 1994 Florida telephone survey that suggests the size of an individual raw oyster meal, for lack of a better term. You can see, not surprisingly, 6, 12, and 24 are the most common numbers, but I don't have a complete distribution. I believe the largest number eaten in one meal was about 50 oysters, which was pretty astounding to me.

We're going to base an assumption on the size of the oysters to go from the weights of the landings to the numbers that are actually consumed. I have here a mean of 20 grams with the standard deviation. This is preliminary--I mean 20 grams is the standard USDA portion of an oyster.

So these data may change by the time we get to the final model.

And, as I promised, I will not keep you from getting your coffee. Are there any questions or does anyone have an assumption that I may have left out? This is the most factual section of the model, I would say. Not a lot of conjecture here.

CHAIRPERSON WACHSMUTH: Any questions? Stephanie.

DR. DOORES: I'm not sure this is for you or whether it's for any of the other people, but were the oysters that were consumed in these outbreaks previously shucked and sold in containers, or were they at raw bars and shucked as they were eaten? Is there any information on that?

DR. DiNOVI: I do not know that. Does anyone know that?

DR. DePAOLA: The data that we have is from Washington State, supplied by the Department of Health, and I think over 90 percent of the cases were due to shell stock oysters there.

CHAIRPERSON WACHSMUTH: That means not shucked.

DR. DePAOLA: Not shucked.

CHAIRPERSON WACHSMUTH: Mel.

DR. EKLUND: Yes. In the outbreak, I think of 1997 and '98, and maybe Marleen will correct me if I'm wrong, but I remember one of the outbreaks occurred during a brunch where they served them about seven in the morning until about noon or one o'clock, and knowing the short generation time of the Vibrio, it's quite conceivable that without ice that it could have reached very high numbers by the time somebody would eat them.

DR. DiNOVI: Yes. Actually, since--I'm going to stick to the factual part of it, but the other is that kind of a factor can be taken into account in the model. I mean you're assuming some variability of the times and lengths and the temperatures. So that can be taken into account.

CHAIRPERSON WACHSMUTH: Peggy.

DR. NEILL: Are there regional--Peggy Neill. Are there regional differences in consumption patterns?

DR. DiNOVI: Yes. Most--well, actually the 1998 and the projection for 2000 actually shows that it's fairly similar numbers in the west and in the Gulf but much lower numbers in the Atlantic and the North Atlantic.

CHAIRPERSON WACHSMUTH: Are you talking about landings?

DR. DiNOVI: Landings, yes.

CHAIRPERSON WACHSMUTH: What was your question, Peggy?

DR. NEILL: I'm talking about eating.

CHAIRPERSON WACHSMUTH: Eating.

DR. DiNOVI: How much people themselves eat? No, I don't have any data to suggest that there are regional differences. There may be data to suggest it, but I haven't seen it.

CHAIRPERSON WACHSMUTH: Bob.

DR. BUCHANAN: It appears that you're making an implicit assumption in your evaluation here that oysters that are landed in a region are consumed in that region.

DR. DiNOVI: Yes, that's true.

DR. BUCHANAN: Okay.

CHAIRPERSON WACHSMUTH: That doesn't always happen. Any other questions? Jeff.

DR. FARRAR: I think you may need to look at that assumption a little bit closer. I think there may be some regional differences there in where the oysters come from.

Secondly, there may be some California behavior risk factor survey data. I hope you have access to

it. If not, I'll be glad to provide it to you on consumption of oysters.

DR. DiNOVI: Thank you. I would appreciate that.

DR. BOWERS: I would just like to add that our output on the simulation is going to be predicted risk distributions, and we'll use the consumption data to estimate the number of illnesses attributable to a region for comparison to predicted risk distribution, but our output won't actually be numbers of cases so that we don't actually have to determine what percentage of the harvesting is within a region.

CHAIRPERSON WACHSMUTH: Okay. The consumption data have one anecdotal piece of information related to cholerae. I know when I was at CDC, we had a cholerae case that occurred in Atlanta, Georgia, and the patient had consumed raw oysters at one of these bars in town and had eaten 30 raw oysters.

DR. DiNOVI: I can always give anecdotal evidence.

CHAIRPERSON WACHSMUTH: Well, I'm sure.

DR. DiNOVI: I don't eat any. There is a large variation. I remember in May someone presented data showing 109 oysters consumed at one sitting. So that number

can be worked in. I mean it would just depend on the distribution.

CHAIRPERSON WACHSMUTH: Okay. We're right on time. Let's take a break, and we'll have some coffee and some oysters.

[Recess.]

DOSE-RESPONSE

CHAIRPERSON WACHSMUTH: Okay. Next is dose-response, Dr. Donald Burr from in town. Don.

DR. BURR: It probably took us longer to get here. Thank you.

Again, we're getting toward the end of the modules, and we certainly appreciate your attention up to part. In this particular subsection of the model, we're going to be concerned with information that is available for supporting quantitative modelling of a dose-response relationship for Vibrio parahaemolyticus. So we'll try to evaluate the relationship between dose and clinical illness, and hopefully during the course of this experiment, several dose-response models will be shown and described and up for discussion, and as Andy pointed out, we're all glad that John is here behind us.

In terms of a dose-response relationship, what we're talking about here is the length between exposure of

the food and outcome. So what we're looking at, we'll try to relate levels of Vibrio parahaemolyticus ingested with the frequency of adverse health effects.

Now, any model must take into account what are the end points that are going to be looked at. At the present time, we were going to look at the model that would consider gastroenteritis alone versus more severe disease including septicemia. As we talked about in May, the prediction of illness--and this has probably a repeat of some of the presentations in the previous days--it is a multi-factorial process that involves interactions between the pathogens and the host and the environment. So this makes it have a very complex function to predict, and that brings along with it a lot of uncertainty as we'll point out as the talk continues.

In terms of data, in order to get dose-response information, essentially there's four areas in which information could be available that we could use. The first one is epidemiological investigations, and this sort of terms accidental or unnatural infections where you have exposure outbreaks resulting in illness.

Now, for Vibrio parahaemolyticus, Marianne has touched on epidemiology does not provide very much data in terms of giving us a dose-response for Vibrio

parahaemolyticus. There's some indication through some of the recent outbreaks that approximately 10 to 5 or 10 to 6 may be capable of produces disease.

The second one is combining epidemiologic and food survey data, and, again, questions here we'll defer to Dr. Buchanan because this is, again, in a paper that he described for Listeria where essentially you combine observed illness with observed exposure and then estimate dose-response relationships from that. Again not a lot of information for Vibrio as you've seen during the course of the last talk.

Human clinical feeding trials, these are controlled experiments with carefully quantitative doses of pathogens in healthy volunteers, and this is what we'll spend time on for the remainder of the talk, and I'll touch a little bit on, also, you can use surrogate models in humans and/or animals, and this is the use of other pathogens as proxy organisms in human feeding trials or animal models for extrapolating dose-response estimates back to humans.

As I said, we'll concentrate now primarily on human feeding trials for Vibrio parahaemolyticus. To date, there has been five clinical feeding trials using Vibrio parahaemolyticus. Three of them were with KP-positive

strains, and two were with KP-negative strains. In the two trials with KP-negative strains, doses as high as 10 to the 10 bacteria produced no illness in the volunteers.

Now, although we have this information and certainly it's what we're all looking for, feeding trials come with a great many uncertainties, both for the host and the pathogen. In the host, we're dealing with a very select test population, generally very healthy adults, generally a very small number. Usually the inocuin is administered after the stomach acidity has been neutralized or it's given in a bicarb buffer or something, and we don't know a lot of about food matrix effects, because, again, you're not getting it in any type of food generally.

In terms of the pathogens for this these particular studies, I think the last study was in 1974. So for the most part, they're uncharacterized strains. They don't--although they're set as KP-positive, we don't really know what the virulence is. We don't really know precisely how they cultured and developed that organism and what effect that would have on the infectious dose. So there's a lot of uncertainties that come even though we have some of the challenge organism and the challenge animal that we want.

This is a graph of the three human trials, as I said, and the first one started in 58, and I think there is one point--let me just make sure. There is one with a dose with a ten to the ninth dose. Sanyal actually had three doses, a 200, 10 to the 5, and a 10 to the 7, and Takikawa had two doses. So again, you're getting a real large range of doses, and as I said before, there's a small number of volunteers that were in each study. But it is possible to take this data and by plotting the probability of causing disease, we can get a dose-response curve.

For our purposes, since, as I said, it's been almost beyond 20 years since these have been done, for our purposes, it would be nice if we could overcome some of our uncertainties by being able to take a new group of volunteers, spike oysters with the strains of interest that are current outbreak strains, and then essentially do another dose-response. Unfortunately, that's not going to happen.

There have been reports of sudden death associated with infection with Vibrio parahaemolyticus, and this is probably also--there was reported cardiotoxicity of the thermostable direct hemolysin. So it's very unlikely that we're going to be able to go back and actually repeat some of these studies with Vibrio parahaemolyticus itself.

So that brings us back to, in order to gain additional information, it may be possible in the future to gain additional information by going to some of the human feeding trials with a surrogate organism, and this may be like Vibrio cholerae 901, actually even Campylobacter. There are animal models with Vibrio parahaemolyticus. There's rabbits, mice, monkeys, and there's animal models with the surrogate organism.

As I said, we've decided at the present time to use the human data, but as we talked about that these models and these risk assessments are a fluid document, that further--as we get more information, these can be used to further refine the model down the line.

Many of you are probably familiar with the disease triangle, and here is just another representation of that, and this is, again, as I talked about previously, in order to produce disease, first you have to have these interactions between the host itself, the environment, and the pathogen, and it takes that combination of the three in order to produce disease. So because of this, just like in the other modules, we have a large many of uncertainties which causes us to make a large number of assumptions which we'll now proceed on to.

In terms of Vibrio parahaemolyticus itself, and certainly this is what's been talked about this morning, there is a lot of uncertainty as far as are the strains of the serotypes equally virulent. We talked about that in the west coast there's a preponderance of certain serotypes. In the outbreaks, there's again certain serotypes that we've seen. This information is not really known because there's also not a lot of animal models that we can really do evaluations of virulence in these strains.

We've talked a lot about known virulence factors, and certainly the TDH stands out as the clear front-runners in terms of virulence determinants, however as mentioned, there are strains--there have been cases--excuse me--of TDH-minus isolates, and it's unknown are those the ones responsible, or does that have to do with our culturing, and we just didn't get the positive one out. So there remains a question of are there strains that are not--are there stains that are TDH-negative that are pathogenic and responsible for disease.

Marianne might have pointed this out, but what are the relative contributions of other virulence factors? We talked about urease, shiga-like cytotoxin, enterotoxins, and enteroinvasive. So there are other determinants that are out there that do at some point have to be considered.

In terms of the Vibrio parahaemolyticus, there are assumptions that we're going to make and starting out that all pathogenic strains are equally virulent. We're also assuming that the human feeding trials provide dose-response data with strains with equal virulence to the current clinical isolates that we're seeing today. The initial simulations will define virulent strains as being TDH-positive, and this is what we've talked about all along, but sort of back to the question that was raised, future simulations may include an additional factor to account for the contributions of other virulence factors as we get more handle on the data for those.

In terms of host uncertainties and assumptions, the uncertainties are: Basically, are certain populations more susceptible to Vibrio parahaemolyticus infection, and as Marianne pointed out, there doesn't seem to be any one particular group that's more susceptible to Vibrio parahaemolyticus in any particular one way, but our assumption is in that the epidemiological data provides data that approximately seven percent of the population with an underlying condition can be predisposed to infection. So this is sort of a general figure that we're using, and overall the general figure in the population for underlying medical conditions that predispose to infectious disease

illness, and we're going to use, as I'll present on the following slides, CDC data that we'll be able to use that implies a crude risk for susceptible individuals about six-fold higher than for general population.

This is recent CDC data, and Marianne published or Marianne presented a large series of data. This is a subset that the CDC has provided to us in which case it comes from Vp Gulf coast surveillance system, and the reason it's a subset is because the details of that information are much more known to them, and so it's a much stronger data base that they have. They have 107 culture confirmed ingestion acquired cases, and this is, again, from '97 to '98.

Septicemia, five of the 107. All were hospitalized, one death. Of four reporting out of this five, three had underlying current conditions. In the remaining 102 cases of gastroenteritis alone, of the 90 reports that came out of that, 27 or 30 percent are hospitalized. There was one death. That goes back to the one death in the septicemia, and of 79 reports, 29 percent had underlying conditions.

Now, they described the underlying conditions as--I've got to get this right so I don't confuse the issue. These include liver disease, alcoholism, diabetes,

malignancy, renal disease, immuno deficiency, blood diseases, gastric surgery, and heart disease. So it's a variety of underlying conditions that they include within this group, and they also estimate that for every one culture confirmed case, that there is likely 20 other illnesses that are out there that go non-reported, and that's the number that we'll use in our models.

By using this data, this slide just shows an example of the calculations that can be used to then determine conditional probabilities of illness and severity for Vibrio parahaemolyticus illness based on the CDC data. Now I need to point out here, I think we have not been the best at naming this. When we talk about susceptible here, and you'll see it in the later slide, susceptible and non-susceptible, we don't really mean--what we're really saying is more sensitive population.

Okay. So when you see in later slides, and I know for some of John's slides, when you see susceptible or non-susceptible, it really means that seven percent of the population having a predisposing underlying disease that gives you that increased risk, so have probability of illness if healthy is dose-related. The probability of illness if you have underlying disease is dose-related with

approximately six-fold lower ID50 than for the healthy. So essentially we'll be able to shift the dose-response curve.

The number of hospitalization given culture confirmed, .3, probability of septicemia given culture confirmed, .05. So again, these numbers we have, and we'll use these as we do our modelling for the disease and the different clinical outcomes.

This is an example of John's calculations for the relative risk for the susceptible versus the non-susceptible, and again using the mathematical models, this is where he has arrived at his number of approximately six-fold. And this just indicates that the probability of culture confirmed illness is not needed to calculate the relative risk in that because as the formulas work out, this just kind of falls out in the calculations.

Now turning to environmental uncertainties and assumptions, again the uncertainties are what effect does the environment have, and it has been mentioned what effect does actually even the oyster itself have on pathogenesis, and again not much is known. There are studies that TDH production is enhanced by the bile acids, and these are bile acids that are present within the intestinal track.

So what role does that have in perhaps turning on genes and turning on virulence? Several animal studies have

shown that acid adaptation enhances virulence, animal passage itself enhances virulence, and what role does the food matrix have in infectious dose? Many studies have shown or some studies have shown with cholerae, by introducing the organism in cooked rice as opposed to introducing it in buffer, you have a lower infectious dose.

So our assumption then is that from the human trials that the growth and delivery of the strains that are reported in the human feeding trials, that we're going to assume that the dose response of that is equivalent to that which you would get in a natural infection, again because we just don't have anything else to base our information on.

This simply brings us up--sort of a repeat to what Marianne had shown early in the presentation. It's a input-output distribution structure of the public health model, and essentially the take-home message is that we're getting to the end here. The arrows are starting to narrow down, and unfortunately for the person who is in this circle, it means that that bacteria was able to navigate through all those other lines and finally get to this point.

But as Marianne said, all the other modules or the two other modules are feeding into consumption and gets us here with dose-response models into our predicted Vp in humans. So here's, hopefully, sort of our end of the trail.

So as I mentioned before, and this will be the approach for modelling for risk of illness, we'll assume that approximately seven percent of the population with an underlying condition is predisposing to the infection. The dose-related risk of gastroenteritis for healthy individuals would be extrapolated based on the Sanyal and the Takikawa data, and the reason we're using those two is because they're the only two that actually had dose-response data. If you remember the third trial had just one dose, and it was ten to the ninth and 100 percent attack rate at that point. So we're using those two.

The dose-related risk of gastroenteritis for susceptible individuals--and again this is maybe sensitive--extrapolated from fitted dose- response in healthy individuals and the differences in the crude risk which I talked about. And here it is, we're using CDC Gulf coast surveillance data to provide information on developing that six-fold higher risk.

This is an example of several different risk assessment, dose-assessment models that are available to determine the best fit for the dose-response data that we have. Okay. So again five are listed, and I think the assistance that we're looking for is, you know, which model is felt to be the better one. Each one perhaps provides

different answers, and as I show on the next slide, Dr. Buchanan keeps emphasizing transparency, transparency, transparency. Okay. If you change the scale of the same models and go to a long scale on the probability of illness, this is the same three or the same five graphs from the previous slide, and if you go back to this slide, around here it looks kind of nice. They all seem to be somewhat similar, but as I said in the transparency, when you change the scale, you'll see that different models produce different results when you get down to the low-dose extrapolation, and those differences can mean a large differences in the outcome that we have. So that's something to keep in mind.

Finally, this just shows using one of the distribution models, the exponential one. This will be the approach to scaling by the six-fold, the extrapolation of dose-response from the healthy to the more susceptible population by using our six-fold estimate.

And finally, I just want to thank--we all want to thank John and particularly Marianne for putting everything together, but also Mahendra, Wesley, and also our CDC colleagues that provided the epi data, Mary and Fred. Thank you.

CHAIRPERSON WACHSMUTH: Thank you, Don. That was very nice.

A few questions at this point? Jeff.

DR. FARRAR: Regarding your estimate of approximately 20 illnesses for every culture confirmed case, there are studies with other outbreaks that show much higher numbers, from 50 to 100 illnesses per culture confirmed case. Can you comment on that?

DR. BURR: This was basically we took this out of the recent CDC emerging infectious, their latest recommendation. Now, I think you're right. This is their data. I think it would be important if there's other stuff out there to bring that in to bear because maybe it's regional.

DR. BOWERS: I think that paper, they looked at lots of different pathogens, and they recognized the fact that there's different reporting for different types of pathogens and Vibrio. Vibrio other was classified as a moderately severe type of pathogen, and the rest of it was there for 20 to 50.

DR. BURR: And I think as we--unfortunately, as we started this model, we were hoping that the epi data would provide a lot more sort of information, and there's

not a lot out there. So certainly if you have more specific information on Vp, that would really be helpful.

CHAIRPERSON WACHSMUTH: Morris.

DR. POTTER: Mary Evans from CDC is here and has confirmed that the multiplier information is in the recently published paper from CDC on food-borne disease. That's available at CDC, the emerging infectious disease general web site and in fact is here. We can make copies for the committee.

CHAIRPERSON WACHSMUTH: Okay. Is it to this point--Stephanie?

DR. DOORES: I just have a question. On those last two slides that you showed of the models, what decisions do you use to decide which modelling program you're going to use for these data?

DR. BURR: Well, I think John, if you want to address it, but that's again the input that we'd like from the committee too, is rejections of--you know, which model is really felt to be the appropriate one. We tried to present all of them that were up there and just make the point that by using a different model you can get much different results when you get down to the low dose extrapolation. So the question is which one is the best one to use.

CHAIRPERSON WACHSMUTH: Bob, did you want to answer?

DR. BUCHANAN: Just a follow-up on that comment. All five models, as far as I know are non-threshold models, that is the working assumption here is that the unit of infection is one cell. So you never reach a zero for a threshold in this instance.

CHAIRPERSON WACHSMUTH: Okay. Dane.

DR. BERNARD: Thank you. Dane Bernard. Don, thank you for your presentation. It's quite interesting as always. I had a question about the assumptions, your slide on uncertainties. You're using seven percent of the population with underlying conditions. Is this, from CDC, now a general assumption as to the susceptible population, or is this specific Vibrio parahaemolyticus in oysters.

DR. BURR: We had--basically the number came out from--it was sort of an FDA internal review of determining that number, but then it was passed through the CDC when we had a conference call with Fred and Mary--maybe Mary would like to comment--that although they didn't really have the time to actually look back to all the data, they're comfortable with that number as well as a general number.

So I'm not sure if I can--I think where Carl got the information--John if you can give a hand--was just

basically on his literature review of his information. So that's a little foggy answer, but we did run it through our CDC folks, and they're comfortable with that number, and we'll firm that up.

DR. BERNARD: I guess we could put it to a vote here and see if we're all comfortable with it, but I was just curious. Since the cases of Vibrio parahaemolyticus seem to be more with white males, the general assumption is that we have kind of a risk-taker population at any rate, and I'm wondering if the number, while it may represent a general population number, might deserve a second look basis of what seems to be the population that tends to get hit most by this syndrome.

DR. BURR: So we should narrow that focus down.

DR. BERNARD: It's just a question. It may be the same number, but I'm wondering if there might be some different way to sharpen the focus on that.

The other question was regarding the six-fold higher level infectivity for that population versus general population, and again where that assumption stems from.

DR. BURR: That was using the CDC data for the incidents. We take those probabilities from that case study and put them--just plug them into a relative risk

population. Is that right, John? And that's where that number comes out, separate from the seven percent. It's not connected to that seven percent. Right? John, why don't you--

DR. BOWERS: Yes, it does connect to the seven percent. What we're using in that--what was shown in that slide was a use of base theorem to convert conditional probability of--see what the CDC data is, it's the probability of seeing--of being susceptible, part of the susceptible population given that there's illness, and we want to turn that conditional probability around into probability of illness given the healthy or susceptible state, and that's just a basic use of base theorem to do that.

CHAIRPERSON WACHSMUTH: Okay. Michael Groves, then David.

DR. GROVES: Mike Groves. I had the same comment as kind of Dane's, is that I think there's a general feeling, general knowledge about people who eat oysters that if you are immuno compromised, you shouldn't do that. I mean I think you'll find that you can't say the general population--you know, transpose that into people who actually eat oysters because there is some education that's gone on, and I guess that affects your relative risk because

your denominator there would change too for the people who are actually eating oysters who are actually immuno compromised.

CHAIRPERSON WACHSMUTH: David.

DR. ACHESON: Just to come back to Dane's point, I think I understand the logic of why you're saying that immuno compromised, for want of a better term, have a six-fold higher risk, but how do you translate that into the previous slide, into dose? I mean you're saying that the ID50 is six-fold lower in these people. How is that translated to dose?

DR. BURR: We're saying that we can shift. I think that's the best way of putting it, that we can shift it down.

DR. BOWERS: I think I see what you're saying. Yes, all we can do is shift--all we've done is we've shifted the ID50, and we've left the slope the same, and your comment might make the point that perhaps the slope of that curve changed.

DR. ACHESON: Right.

DR. BOWERS: You're right.

CHAIRPERSON WACHSMUTH: Any other questions? Thank you, Don.

INTEGRATION OF ALL MODELS

CHAIRPERSON WACHSMUTH: The next presenter is Dr. John Bowers who will talk about the integration of all the models.

DR. BOWERS: My name is John bowers. I work in the Division of Mathematics in the Center for Food Safety and Applied Nutrition, and I'll be presenting really an overview of what's already been presented this morning, and I will be just focusing in more detail on precisely how we propose and have worked on structuring our risk assessment model, and I'll be going into some of the details of how we plan to--the mathematical details of how we plan to build the simulation.

I first just want to say a few words about Monte Carlo simulation to set the tone for discussing each of the modules in turn. The Monte Carlo simulation is a representation of variation. We choose inferential parameters determining the ultimate output trend that we want to predict, which is risk, and we have to model these distributions, the modelling distribution of variability, and we'll also consider uncertainty in our specification of the model.

By variability, I mean parameters such as temperature. This is a real variation, we can see this. Uncertainty would refer to things like percent pathogenic or

perhaps, you know, within a given reason or season this is fixed, but we're uncertain as to what that exact percentage is, and we need to evaluate that uncertainty.

We have two types of distributions. This actually refers to how we go about constructing these distributions mathematically. With temperature, we can just look at the--we have the observable data right there, and we can choose an appropriate representation. For other parameters in our model, we have to use correlative relationships to move from distributions that we know to distributions that we can't see very well. A good illustration of this would be the distribution of pathogenic vibrios.

Why representation with distributions? Well, there's a real variation in the environment. We know that. We've already built a credible risk assessment. We cannot ignore variation. We have to model it. Consideration of variation about mean levels gives an indication of the frequency of extreme events, high Vibrio levels, perhaps this is the cause of a high proportion of the illnesses.

Mitigation efforts may change the variance as well as the mean. A mitigation which lowers the mean but increases the variance might not be effective, and you can't

look at the--you can't even address this unless you build a Monte Carlo simulation of the variance.

Our model scope, this is just our three models of environment, harvesting and storage, consumption, and susceptibility. In the environment, we have regional, seasonal, and year-to-year variation in the parameters to be considered. Temperature distributions vary across different years. They're not all the same, and we want our model to reflect that.

Harvesting and storage, Andy has gone into this in great depth, and I'll just touch on some of the points that he's already gone over. We have regional and seasonal variation and length of the harvest day to be considered and incorporated into our simulation. And certainly with consumption and susceptibility we have two populations to consider, even though we perhaps don't know what the right percentage of the population is susceptible.

The mathematical process or the process of building our simulation, we have to choose the appropriate mathematical presentations. We have lots of distributions to choose from, lognormal, normal, Beta-PERT, uniform, triangle. We have to determine the appropriate mathematical relationships which we are using to go from one parameter,

such as temperature, to concentration of Vibrio. We use regression and nonlinear models to do this.

This is just a summary of the distributions and the relationships that we're using and going to use in the construction of our simulation as we propose to do at the present time. On the left, there's the distributions, the water temperature, the salinity with a star next to it, and I'll get to that in the next couple of slides. We have the total Vp concentration in 12 oyster composites, and the ultimate distribution we want to predict in this module is the concentration of pathogenic Vp.

Relationships, Marleen has already shown you the regression relationship that we have developed based on Andy DePaola's data from 1990 with both water and oysters, and she's also presented some of the statistics of percent of isolates pathogenic which will ultimately be our basis for moving from the distribution of pathogenic Vibrio to--excuse me--moving a distribution of total Vibrio to the distribution of pathogenic Vibrio.

This is an influence diagram of the relationships between these distributions, and we kind of think of this as moving from the top down to the bottom. At the top, we have what I would call upstream distributions, and at the bottom we'd have downstream distributions.

Regional: In this particular module, we have regional and seasonal and yearly variations is driving distributions which in turn cascades down to pathogenic Vibrio distribution. I really just added this more for completeness. All of these choices, on the left there's our distributions, and on the right are mathematical representations of them which are not completely fixed at the present time, but I don't think I'll going into the detail much on that except at the bottom, the pathogenic Vibrio distribution, based on figures such as .4 percent pathogenic or .3 percent pathogenic depending on the west coast or the Gulf, that kind of centers how we can use that data to get an idea of how to shift down, take the total Vibrio concentration distribution and shift that down probably two to three logs. But we're quite uncertain on how the shape of that distribution changes, that is the variance, and that's an uncertainty that that we'll have to evaluate.

This is just a picture of water temperature distributions, just to show you that there is variation there. The dark line there is the median. This is Dauphin Island in the winter. You can see the medium is changing. You can also see that the variance is perhaps changing a little bit too.

This is getting back to a comment that Bob Buchanan brought up earlier here, how we best deal with salinity in our model. We have very limited data on salinity. Most of our data that we've been able to compile so far is Washington State, the state shellfish people, I believe, some data from Texas, Galveston Bay, and some data from the Chesapeake, but we know that salinity is highly variable within a given estuary. I mean up river in the estuary versus lower down towards the ocean, and there is a question about how representative this data may be of the salinity that the oysters are truly living in.

So we ask ourselves the question is it necessary to model salinity effects. A regression relationship that we've developed suggests that there's a relatively small effect in the 20 to 30 PPT range, and the other piece of information that we have is that the ISSC/FDA retail study suggests that oysters are infrequently harvested from waters outside the 20 to 30 PPT range. This is based on looking at the salinity in the oyster, and there is independent data showing that this correlates very well with the salinity of the water in which the oysters are harvested.

And this is just a picture of that. What I've done is I've input a fixed temperature and drawn a curve of

predicted. This is based on a regression equation that was fitted. I've just plotted the curve of the relative amount. As you move away into higher or lower salinities, the equation predicts less Vibrio, and how much is that difference relative to the optimum, and the answer there, I have just a box plot of the distribution of salinities from the ISSC retail study, and you can see that it seems to indicate that 20 to 30 percent are in the--that most of the observations are in the 20 to 30 PPT range, and the effect of salinity, at least as presented by the model, would be a very minor effect, perhaps five to ten percent.

Again, this is just for illustration. This is salinity in the summer in Washington State, just to give you that the variation--there is year- to-year variation, and we'd like our model to depict this type of variation and cascading down to predict how the distribution of Vibrio varies from year to year.

I believe I've already gone over this. This is just a recap of what Marleen was talking about on the percent pathogenic and how we would use this information to infer distributions for pathogenic Vibrio, but we're very uncertain about what the change in the variation of that distribution because we can't really assume it's the same as for total Vibrios.

And this is just a picture of our output from the model. As we have it right now, I've just inputted mean temperatures, chosen typical salinity levels. They are 15 for all levels except the west coast. There are higher salinities there. I would just like to point out that our goal is not just predicting the mean well. We're always interested in the variance about the mean because those two things constitute the distribution, and our estimate of that is at the present time based on the regression is--for the variance above, the regression line was 1.0, but we know that that's both natural variation of method error. Unfortunately, we don't really have an accurate estimate of the method error for this membrane filter method, but we know it's less than .35 or we're relatively confident that it is.

Validation, at all points we'll look to validate for this module. There are studies of Vibrio levels at harvest, various types of studies, vulnificus studies. They're measuring salinity and temperature. We compile that data, and we can compare that against this National Buoy Data Center data that we've compiled.

Issues and limitations: We have no account of the effect of nutrients, oyster physiology, chaotic events such as seeding by ship ballast. The effects of salinity

may be important, but the salinity profiles are different to determine, and we would welcome any comments that you would have. And probably most important, we have an uncertainty as to the relationship between pathogenicity to total Vp.

Again, these are all the distributions. I won't go through them in detail. I'll just--for your reference, this is, again, an influence diagram. At the top left, we have the distribution of Vibrio levels at harvest, and really this picture is just cascading down to Vibrio levels, distributions at the time it reaches cooler temperature, and then later at the time of consumption as these distributions are influenced by time to refrigeration, the air, temperature, the cool-down time, and the length of storage time.

I would just like to point out that in the simulation, as we propose to construct it, we're going to take account of the correlation between water and air temperature because these tidal basins are very shallow and they mix very well with the air, and you can look at the data, and there's a very nice correlation. The water temperatures in our simulation, as we generate random samples, we'll pair up the water temperature, random samples, with the Vibrio levels they predict, and then we'll

use a water/air temperature difference to get the right air temperature, the appropriate air temperature.

Again, this is check for your reference, the distributions--the mathematical representations of the distributions that we intend to use at this time--propose to use at this time.

The ambient air temperatures, these are just two examples in the Gulf coast in the summer versus the west coast in the summer. On the west coast, the air temperature is generally--on average, it's warmer than the water, whereas in the Gulf coast in the summer, it's the reverse, and so as we're building the simulation, we'll have paired water temperatures predicting Vibrio levels, and we'll use relationships like this. It's a mean and a standard deviation, and we'll just adjust the water temperature to predict the air temperature, and that air temperature will in turn be used to predict the extent of growth that may occur postharvest.

Andy has gone into this in great depth. He's already shown you this graph. This is not the time that oysters are left unrefrigerated. It's the duration of the harvesting times, harvesting days, and it's skewed over toward the right because most people, when they go out to harvest oysters, they're going the put in a long day. We'll

assume that the oyster lots are harvested uniformly during the day, and we'll--actually, the simulation will be run by randomly sampling from this Beta-PERT distribution and then randomly sampling uniformly between the time of one and that time of harvest.

Our growth, this is the prediction equation from Miles, et al., actually for temperatures of practical concern. We can really take an linear approximation of this, and it will work just fine; and as Andy has already said, the growth rate in the oyster at 26 degrees is one-fourth that predicted by this equation from the broth data, and as we evaluate the uncertainty in our predictions, we will vary the one-fourth factor in order to see whether that has undue influence on the output.

The model of the Vp growth during cool-down, we've proposed to simulate a time to no growth condition as something uniform between about a half an hour and ten, and that the growth rate falls linearly during this cool-down period. So you can sort of think of this as a kind of a--we'll do this as a discreet simulation so that the rate of Vibrio growth is just kind of ratcheting down in discreet steps towards zero, but we will ensure that at no point would it be able the exceed the maximum level of about 3.1 log growth.

Storage time and die-off, we use a Beta-PERT distribution mean of 7.7 and a minimum of one and 28 days. That's kind of skewed over to the left. There are not very many of them out there at 28 days, and the die-off is--our estimate is .07 log per day.

We don't have any output, but if we did have output to show you, we'd have a cell with a table, 12-by-4 table with 16 cells, and in each cell we'd have a predicted distribution without any mitigation, with the mitigation effects that we're evaluating, freeze and heat treatment, and we would iteratively drive this model through the environment to see an indication of how these distributions vary across different years as the temperature and salinity perhaps vary.

Our validation in this module is the ISSC/FDA retail study, and this is just a graphical picture of what Andy has already presented, and I would just point out that from this data we are also getting an estimate of the variation about the mean, and we want an estimate of variation about the mean because our model is predicting both the mean and variation, and our estimate based on the data is about 1.4 standard deviation at retail.

Limitations, Andy has already gone through this. It's the growth rate of pathogenic Vp is assumed to

be the same as for total Vp, and in growth rate versus temperature, we do have an uncertainty in the relative growth in the broth versus oyster that we need to evaluate and make sure that doesn't unduly influence the simulation.

All right. In the public health module, our input is the concentration of Vibrio consumption. In 12 oyster comps, it's because this is the way these studies are done. We know that people don't uniformly eat 12 oysters at a time, although probably frequently so. We'd like to adjust that distribution to distribution in single oysters, and the distribution in single oysters is likely to be more variable since the process of taking 12 at a time is an averaging process, and we have to consider the number of oysters per meal, the oyster weight in order to calculate the total dose per meal, and then we have to move on to the risk per meal, and our relationships are pretty much the ones that Don has already given.

And this is the influence diagram for these distributions in the previous slide. We start at the top, and we cascade on down to the bottom, and at the bottom, there I have probability of risk for random selection of doses, D-1 to D-N, and we're actually going to evaluate several different models.

We use consumption statistics to validate our risk per meal predictions. Regarding an earlier comment during Mike DiNovi's talk, we would like to know the percentage of meals in the Gulf coast that are consumed locally, because some of the best epidemiology or the epidemiology that we're most comfortable with is from the Gulf coast, and we'd like to estimate the number of meals or eating occasions in the Gulf coast and then use the surveillance data there to get a--as a validation point for our model but perhaps not necessarily for the other parts of the U.S. if our goal is simply the total risk in the population, not just regional risk.

Again, this is a slide showing the mathematical representations of the distributions. Our dose risk calculations, we'll simulate random samples of the number of oysters eaten per meal. We'll randomly sample the weight of the oysters and multiply that out by the estimated distribution--by random samples from the estimated distribution pathogenic Vp. That will give us random doses, sets of random doses, and this will vary. We'll do this for each region and season, and we'll get a distribution of risk by using--just transforming the dose according to the mathematical dose response relation, and we'll evaluate several different models there.

This is just a picture of the Florida data that Mike was talking about. The insert is the box plot of the oyster weight as I was calculating it based on this particular data. It's a little bit higher than 20.

Don has already presented this, but I think you can see, I mean, based on a .4 percent pathogenic--I'm just doing some mean calculations. The total Vp dose--the pathogenic Vp dose per meal is in the range of about two to three, maybe three, and you can see that just looking at the bottom log, dose three, and moving on up, you can see that there's a considerable variation of risk there, predicted risk.

This is really just slide going over what Don has already presented, and I think he did a pretty good job of it. It's just seven percent of the population with an underlying condition. We're using the CDC Gulf coast surveillance data to derive a relative risk. This is the mathematics. This is the mechanics of how you calculate relative risk.

In the CDC data, you can look at the percentage of the cases that are being captured which have a health condition versus not, and if these health conditions had no influence, you would expect that seven percent of the cases captured would have one of these conditions, but in

fact we see 30 percent of the cases that the CDC said have an underlying condition. So this indicates that there is some elevated sensitivity here.

We can use the same mathematics to calculate the probability of progressing on the severe outcomes such as septicemia and death, and on the last three bullets here, the third from the last, the probability of septicemia given that you're healthy is two in 100.

Then you go on to the next one, the probability of septicemia given that you're part of the susceptible population is four percent of the population, that risk is tenfold. This is what is CDC data indicates.

Our validation point for this module is the same for sporadic illness data in '97 to '98, and these are the non-outbreak cases broken down by season. Divide that by two to put it on a yearly basis and estimate the number of meals in each season to calculate a crude risk, an average risk, and that would be something that we would compare the model to, the model output to. And that concludes that.

Issues and limitations: Uncertainty of low dose extrapolation, we have to evaluate that, and I think my second bullet there, possibility of different shapes as well as location, we already brought that up, the results of the

probability that the relative risk is based on culture confirmed cases. It's not total cases.

Thank you.

DR. POTTER: Okay. Fine. Thank you.

Comments or questions from the committee? Dane.

DR. BERNARD: Thank you. Again, thanks for the presentation. Going back to my questions for Don, and this might be way off base, but you're the expert in the statistics and all of that. If we were to have confidence in the seven percent susceptible population consuming oysters--and I think maybe we need to re-look at that because the susceptible population that eat oysters may be a bit higher than that. But let's assume it is seven percent. At the same time, they represent 30 percent of the cases. Is there any information there that would allow you to sharpen your estimate of increased sensitivity, the six-fold increase in sensitivity? It seems to me that we've got some information here that if we have some confidence in those percentages that might give you a tool to sharpen up your sensitivity calculation. It's just a thought.

DR. BOWERS: Well, the sensitivity calculation there is based on an assumption that it is seven percent exactly. If you wanted to make that uncertain, then the six percent becomes very uncertain too.

DR. POTTER: Bob.

DR. BUCHANAN: On the different dose-response models that you presented, are the confidence intervals around each of those different models the same, or do they look different as you go further and further down into the low dose extrapolation?

DR. BOWERS: I didn't look at that. I should have looked at that. That's probably like a big sloth that extends down, and they overlap because all those curves are just the maximum likely estimates based on the data, and the best estimate, that's just a best estimate. So yes, there's probably a good deal of uncertainty there.

DR. BUCHANAN: I seem to remember a discussion by, I think a paper Marks where they looked at the air distributions around each of the different models and came to some conclusions about the adequacy of different models and extrapolating backwards in terms of relative errors, and that might be good to look at before you make your final selection on which would be the appropriate model since you have such a big difference between each of those.

DR. BOWERS: Agreed.

DR. POTTER: Jeff.

DR. FARRAR: Back to a comment you made about the purpose of the model to predict total risk and not the

look at regional risk. I guess I'd be interested in the comments from other committee members, but I feel that regional risk may be an important outcome as well if there's sufficient data to begin to look at regional risk.

DR. BOWERS: That's directed at me?

DR. FARRAR: You or any member of the committee.

DR. BOWERS: Okay. Yes, I agree, provided that--if there's data available to figure out the interstate transport works and the fraction of the harvest that goes different places, then it's worth doing but we don't have it right now, but it could be done.

DR. POTTER: Andy.

DR. DePAOLA: In the retail study, we did keep track of where oysters were harvested and where they were consumed looking at nine different states. So we do have some information as to where they were consumed. We didn't see that it made that much difference if they were harvested on the Gulf coast and consumed there versus if they were sent to Chicago or Denver or Virginia, that the levels were that much different.

I think that we do need the look at the primary factors of where they were harvested and not where they were consumed.

DR. POTTER: Peggy.

DR. NEILL: I just wish Spencer was here to comment to this last point because as I have been given to understand it in some of the previous presentations there's variety permutations in oyster traffic after harvest to the point of consumption, and I recall Spencer speaking of, for example, oysters harvested in the Gulf, depurated off the Virginia coast, shipped someplace else to the distributor, broken down and repackaged etc.; and as I have understood it, there are no hard figures on trying to then look at proportions within these two points between harvest to point of consumption. Maybe we could ask him this afternoon.

DR. POTTER: Once more, Peggy, the last statement.

DR. NEILL: Maybe we could ask Spencer to comment to this last point if he's here this afternoon.

DR. POTTER: Okay. Angela, can you address that at all?

DR. RUPLE: if I understand what you're saying, I think the data from the retail study would be the closest you could come to addressing that, because that study was done at the retail level so that is as close as you're going to come to what is being consumed. So those

concerns--those things do happen, but I think that would be addressed in the results of the retail study.

DR. BOWERS: That's a good point, but there is--there are, I think, 370 samples. So that might be enough to get a crude estimate of how much is shipped from one region to another.

DR. POTTER: What do you think the impact of that would be on the model? I mean is this going to have a major impact?

DR. BOWERS: It would just be breaking the--as I was looking at it, was the output would be a risk per eating occasion in each of the 16 cells that we have. You need consumption in order to turn that into a distribution of the number of cases occurring if you want to do that, and if you want to do that on a regional basis, then, yes, you have to know about regional state transport, interregion transport.

DR. POTTER: Other comments or questions from the committee? David.

DR. ACHESON: I want to come back to this issue of the number of--the ratio between the diagnosed cases and the undiagnosed cases which you were saying the conclusion was 1 to 20.

DR. BOWERS: Yes.

DR. ACHESON: That's based on CDC data?

DR. BOWERS: That's based on a recent CDC paper summarizing the data and their conclusions.

DR. ACHESON: Okay. I guess I should direct my conclusions to CDC as to how accurate they feel those numbers are, because it's not an organism that is typically looked for in a clinical microlab with somebody presenting with gastroenteritis.

DR. SWAMINATHAN: Mary Evans, if you're in the audience, would you like to answer this?

MS. EVANS: I mean, I obviously didn't write the paper. It's based on the burden of illness paper from Paul Mead, and he used Gulf coast surveillance data from, I think--I believe 1988 through 1996 and also FoodNet active surveillance data to come up with those estimates. So I guess I would have to defer the questions about that directly to him.

DR. LANG: Art Lang, CDC. The only thing I can add to that is that what some of the FoodNet surveys allowed Paul to do was to get a sense of underestimates due to various medical laboratory practices so that they've done laboratory and physician surveys on their culturing practices in certain settings, and so the estimates are little bit better than just pulling out of your back pocket.

They are based on some data about practices in the health care community and as well as at the state and local public health level, that all those layers of activity that full through the reports that actually get to CDC.

So I'm sorry I can't get you a more detailed answer but the 20 to 1 estimate.

DR. POTTER: Peggy.

MS. NEILL: I think the data come from two separate studies, one done by Marty Blaser a number of years ago on Salmonella underreporting which found that there was an approximately 36-fold which plays into that number then of about 40 to 50, and so what was done in this paper if I remember correct, the burden of illness paper by Paul Mead was that for those pathogens considered to have a non-bloody diarrhea presentation, they were often adjusted with a 35-fold multiplier for total number of cases from those reported, and there is data for 0157 that is similar and that found a ratio of approximately one reported case for 20 that had actually occurred.

So across a variety of the pathogens in the paper, they've divided them into two categories of presentation, those that are bloody or more severe and used a 20 fold multiplier, and those that were non-bloody or less severe got lumped into a category using the other multiplier

which in turn goes back to the two different data sets. Whether that's correct, I think remains to be seen.

DR. POTTER: If you would like more detail than that, David, than Peggy just gave you verbally, LeeAnne can give the web site citation for the report, and it does go into the details of how the estimates were derived.

MS. JACKSON: If you want the web address, it's listed as:

HTTP://WWW.CDC.GOV/NCIDOD/EID/VOL5NO5/MEAD.HTM

If you want to just go directly to CDC's web site, it's in their emerging infectious diseases publication. You can just access it that way instead of trying to type in the entire address.

DR. POTTER: So CDC.GOV ought to get your there. Mike.

DR. JAHNCKE: Yes. I have just a comment on the previous topic effects and shipment of these oysters. These oysters, if they go whether from the Gulf coast up to the east coast, they do have a harvest tag with them that identifies the point of harvest. I'm sure from some of the state Shellfish Sanitation Departments and things you may be able to get estimates of the--and other sources in various states, estimates of, you know, how many Gulf oysters are

sold up on the east coast or along the western coast also. That can help in your development of your model.

DR. BOWERS: That data is in tabulated in the ISSC study, the origination state and the destination state. So, yes, we can use that and take a closer look at it, and we should.

DR. POTTER: Mark, did you have a comment? Okay. Fine. Other comments or questions for John?

Okay. With that, then we'll go to last presentation of this session.

SUMMARY AND CONCLUDING REMARK

DR. MILIOTIS: Okay. The end is in sight. What I'm going to do right now is see are we on target, have we reached the milestones we set out to reach, have we addressed the questions that the risk assessment was charged with, what the data gaps are that we have identified, and once again we'll enlist your help.

For September 1999, according to the time line, we were supposed to present a draft risk assessment. Have we done that? We have developed a draft risk assessment model on the Vp which we have presented today.

Have we addressed the question, the first question, what is the frequency and extent of pathogenic strains in shellfish waters. We are not certain. As both

Marleen and Andy and everyBODY has said, there is a need to determine what IS the virulence factor determination. We have assumed .4 percent in all the states except the west coast based on TDH frequencies from observed values. Is this correct?

WhAT about other pathogenic strains? What parameters can predict presence? From the outbreaks, it is almost certain that temperature is one OF THE driving forces that promotes growth of Vp. In a Texas outbreak last year, the temperature was significantly higher in May and June than it was in the previous five years for that same time period. If Washington state, the 1997 and 1998 outbreaks were both during El Nino years which indicates warmer temperatures.

What about salinity? The Texas outbreak showed a significant increase in the salinity levels in May or June of last year from the previous five years, however this salinity and the temperatures are the same, are very similar to normal summer temperature and salinity levels. Is it, maybe, then the sudden increase in temperature/salinity that makes the Vp, the pathogenic Vp and non-pathogenic Vp grow quickly or multiply?

Ballast waters: as Marleen suggested, Ballast waters have been suspected and in the Texas outbreaks ships

originating from the Asian countries have passed through the Texas Galveston Bay harbor prior to the outbreak, and 03:K 6 was identified for the first time in the United States and associated with the outbreak, and that's normally an Asian strain.

Marleen mentioned oyster physiology. The immune status of the oyster may play an important part and what tidal flushing and what other factors are. We are looking at these. Unfortunately, temperature and salinity are basically probably the only ones that we can do a quantitative risk analysis. We need to do a qualitative risk analysis on the others.

What is frequency of pathogenic Vp in shellfish? This goes the same as for waters. We are not certain. Once again, we need more virulence--determination of other factors.

How do the levels at consumption compare to the initial levels? Preliminary analysis of the ISSC/FDA retail study showed higher Vp densities at retail than at harvest for the Gulf coast and the Mid-Atlantic.

What is the role of postharvest handling? Vp--right now, I'm just basically repeating what Andy and other people have said, and I'll try to summarize it. Vp multiply

in oysters if they're not refrigerated. The Vp densities increase slowly during the refrigerated storage.

What intervention strategies can be used? As Andy showed, mild heat treatment and freezing markedly reduce the levels of Vp in oysters, and there's also now the question of hydrostatic pressure that needs to be determined.

The question arose about using depuration. That's one of the things that we're going to ask here at the end.

What do we know about dose-response? Both Don and John showed you a dose-response curve. Animal studies which Don had shown you last May and clinical studies have shown an increase in the number of illnesses with increasing levels of pathogenic Vp. How does dose-response vary for the different strains of Vp? Nonpathogenic strains do not cause symptoms in animals or humans, and we definitely saw the dose-response curve from pathogenic strains.

As you will see in our assumptions, we have assumed equal virulence for all virulent strains. How does the dose-response vary among humans with different susceptibilities? There's been a lot of questions and concerns about this, but the dose-response relationship seems to be a six-fold lower ID50 for the susceptible

populations, and it appears to be a greater probability of septicemia and death.

Finally, is current knowledge adequate? We have identified several data gaps which I will go into. Where should future research be directed to reduce uncertainty in the risk estimate?

The incidence, I'll mention a few, and there may be a few that I've forgotten, and please help me if I have. First, the incidence of frequency of pathogenic Vp in water and shellfish, we need more determination--to determine the other virulence factors if they are important. Factors that effect the incidence of pathogenic Vp in the environment, we know temperature is probably one of the driving forces. What about the others?

Again, the role the oyster plays in levels of Vp, we definitely need more research on pathogenic strains other than TDH except and urease and enterotoxins. We need to know the incidence of these factors in Vp in clinical versus environmental isolates, and we need more dose-response data.

We have--as far as I know, we have one published paper on urease, the dose-response or urease in animals. It just gave us the means, no standard deviations or anything like that, so we could not use that in any of

our studies, and we also have one unpublished study which is has been down in our lab showing that urease-negative, TDH-negative strains do produce diarrhea in animals.

Please help. Are our assumptions valid? Are they acceptable? Are they reasonable? Once again, we go to the .4 percent Vp in all regions except the west coast, and based on TDH, do we need to add to this, or can we leave it at .4 percent, especially in the west coast?

The 1997, 1998 outbreaks, all the TDH-positive strains were also urease-positive. So we could basically take into account for the west coast at least 3.2 percent as being pathogenic.

Is our water temperature data representative of the oyster harvest growing areas for each region? Are our time to refrigeration assumptions correct? We've assumed there's similar growth and survival of all Vp in oysters regardless of pathogenicity. We have a cooling rate of .003 of logs for gram per hour. Is that correct? I'm sorry. That was die-off rate. The cooling rate would range from .5 hours to 10 hours. Is that correct?

We've also said equal consumption patterns between susceptible and healthy population, and our virulent strains are equally virulent. Here are time to refrigeration assumptions, the 1996 data representative of

Gulf for the summer, and you have all of these in your handouts. I will not go into all of them.

In air temperature assumptions, are we on the ball with that? Our growth model assumptions, is it correct to say that no matter what temperature growth in the oyster, it is always going to be a quarter of that of broth, and can we say salinity does not effect growth?

Other assumptions, we've said that there's a constant harvest that continues throughout the day. Is this true?

Depuration effectiveness, the question came earlier. There were three studies. Two were done with naturally-infected oysters. One was with lab-infected oysters. It has been suggested that the naturally-infected oysters actually colonize, get within the oyster and colonize the oyster gut, whereas the lab-infected, we don't know how well colonized they are.

And the other assumption is the regional consumption differences. Are there regional consumption differences. Someone mentioned about risk per illness per region or global. I hate to bring a personal touch to this, but a couple of weeks ago, I went to a restaurant, and my husband had an oyster sampler and ate oysters from Washington State, from California, from Maine, from the Gulf

coast, and he had symptoms the following day, very acute, very brief. How do we know which of those oysters caused the symptoms?

Again, please help. Any information you have that can be used to address these data gaps, any other information you have to validate our models or to tell us are we approaching this the right way. What are our mistakes? What can we do that will improve this risk assessment?

Okay. This is a list of our task force members. They all worked very hard to get us where we are today. I would especially like to thank Andy DePaola, John Bowers, Don Burr, and Mark Walderhaug who have gone beyond the call of duty to help us, and finally but not lastly, I would like to thank all the people you see--sorry, Robin, that was cut out a little--who helped us gather all the information we have, and thank you for your time and attention.

DR. POTTER: Okay. Thanks, Marianne. Would you please go back to the slide, about four or five slides back that said please help on it?

DR. MILIOTIS: The assumptions?

DR. POTTER: Yes. Okay. I'm not sure that we need to go around the table one question at a time and get

an opinion from each person, but I think that it would be useful perhaps to have this in front of the committee as we try to think through some of the assumptions and the overall risk assessment, and keep in mind that this isn't the Joint Institute for Food Safety Research priority setting, but rather are these assumptions acceptable for the purposes of a risk assessment for whatever function the risk assessment has, not to perfect knowledge of physiology of oysters or Vibrios or anything else, but for our purposes in food safety.

So are these assumptions acceptable? Here are some of the assumptions. Let's give Marianne and the team that have done the risk assessment some help here. Comments? Mike.

DR. JAHNCKE: I have one comment. One of your assumptions that is not up there--maybe I have missed it--all of your dose-response models and things are based upon those two earlier studies, one done in '74, one done in '59, and you're basing everything on those. I think one of your assumptions would also have to be are there any limitations to those earlier studies? I mean that's the best data you have so you're using it, so, but I don't see any assumption on there indicating are those earlier studies appropriate.

You know, how were they conducted, the

matrixes that were used? I think that at least ought to be identified as one of your assumptions.

DR. POTTER: Okay. But to that point, Mike, is a dose-response assumption based on five or six data points acceptable for the purposes of the risk assessment, or do you think that they go back to the rectangular distribution and assume that one Vibrio is as high risk as ten to the fifth?

DR. JAHNCKE: I think that's what part of their assumption was, was that one Vibrio can be as--

DR. POTTER: No, the point is that there is a--is that more likely in your mind to reflect sound biological science than assuming that there's going to be a same attack rate at any level of Vibrios? I think that's kind of the question we're getting at.

DR. JAHNCKE: I think part of that is based upon the other factors that you identified previously as far as the food matrix, the acidity of someone's stomach at that point, all of that is going to be part of the virulence or the attack rate of the Vibrio parahaemolyticus.

DR. MILIOTIS: Unfortunately, that's all the data we have, but there is a study. We have a grant from the University of Maryland, and they are going to have--I think we've mentioned this before. They're going to feed

human volunteers with Vibrio cholerae 901 that have been infected with oysters. They're going to give these people raw oysters infected with Vibrio cholerae.

In conjunction with that, they're going to feed animal models with Vibrio cholerae 901 and Vp because of the similarities or difference between Vp and cholerae in the animals and extrapolate to the humans, and that way we'll get the food matrix effect.

DR. BURR: I was just going to say the other thing is we do--in May, we talked about the other feeding trials, the surrogates. There are some feeding trials with 901 Vibrio cholerae. There's some recent ones with Campylobacter. The question is do we bring in those surrogates and add that into the model.

DR. MILIOTIS: That's why--unfortunately, the studies are being conducted presently, and that's why Glen Morris' study will be very important, because then we could actually extrapolate the Vp with the Vc in the clinic in the humans, because as Don mentioned, I don't think there's ever going to be a chance of having a Vp clinical study again.

DR. POTTER: Okay. Let's talk to that point then. Alison, something on the use of surrogate organisms?

DR. O'BRIEN: Related to dose.

DR. POTTER: Right.

DR. O'BRIEN: Just an observation for your presentation, Don, or at least what I heard you say. You had a slide on data sources, and then you had a slide showing the different dose-response curves or datum points in the human studies, and if you looked at your fitted curve in human studies for ID50, it fell somewhere around ten to the fifth or so, ten to the sixth from that figure that you had, but you had a comment in passing under epidemiological investigations that the dose was about ten to the fifth to ten to the sixth.

Well, if that is correct, then at least it gives me some confidence that for normal people, an ID50 might be somewhere around ten to the fifth to ten to the sixth. Your passing comment is what I'm asking about.

DR. BURR: Right. There wasn't a lot of epi data, but the recent investigation in Texas did seem to validate what we're seeing in the human challenge models. So you're right.

DR. MILIOTIS: The only thing is in the Texas outbreaks--

DR. BURR: They never--

DR. MILIOTIS: Go ahead, Don.

DR. BURR: In the Texas outbreak, when they went back, they never actually found the virulent strain.

By the time they did their sampling, they couldn't detect it. So, again, that's sort of, I guess, going back and getting things from the past. So the direct evidence is not there.

There's also--I didn't put in--in May, there was some indications that there have been some reports with some laboratory infections where, again, they go back to, well, how much do you think you've got in you, and again the comment was, again, that it was a very low innocuant, again bringing it around to that ten to the five level.

DR. POTTER: Okay. Other comments from the committee on your sense of whether the dose-response curve would be made more robust by adding data from other organisms or using animal data, or any sense from the committee on that? Bob?

DR. BUCHANAN: Just as a general observation, every time you add a surrogate or a closely related animal model, etc., then you wind up going into the issues of how do you correlate one with the other, and you wind up with an even greater degree of variation in your dose-response curve as a result of the degree of uncertainty you have to add back into the model.

So I'm not sure that in the long run you wide up gaining anything by adding this additional data because

you wind up with a broader band that you have to consider in terms of the uncertainty you've generated.

DR. POTTER: So you think perhaps staying with the curve that's generated by the data points that exist at least until the study at the University of Maryland is done?

DR. BUCHANAN: Yes, I would lean towards that as this is the best data we have on target and until the second interration or the third interration or whenever this data becomes available, to focus on the trials we have as long as a good review of the original protocols indicate that there wasn't any gross error in the protocols.

DR. POTTER: Okay. Other comments on dose-response?

Okay. The frequency of pathogenic Vibrios as part of the total Vibrio parahaemolyticus in the environment. John Kuenberg.

DR. KUENBERG: Thank you. I just will bring up what I heard as a sense of the committee, and there was a caution or a caveat relative to including things such as phage to bias survivability of the organism. We've had some hall conversations about that. So if there was a bias, it might be that it's a low number which measure may be higher than what's projected.

DR. POTTER: John, explain to me. I'm perhaps being a little dense here, but are you suggesting the assumptions, they are not acceptable because they should be estimating a higher proportion of the total being pathogenic?

DR. KUENBERG: That's what I was hearing here as discussion at the committee, was the survival selection factors may bias the numbers to be readable lower than they may be. There's no hard evidence, but it's a caution, or I don't know what you would call it, uncertainty perhaps.

DR. POTTER: Okay. Mel.

DR. EKLUND: The Japanese have had a tremendous amount of experience with this, as you know, and the years that I've been in Japan, Dr. Sakazaki has often indicating that when an outbreak occurs they go back to the source, and they can find very few Kanagawa-positive organisms, yet when they find these specimens from a victim, they find a very high percentage of these.

So I think this is something that's very interesting because that may put into the fact--I think even Sakazaki used to say, well, ten to seventh or maybe ten to fifth organisms is required for an outbreak. I think this virulence factor could play an extreme role in the total dose-response.

DR. POTTER: Okay. Bob.

DR. BUCHANAN: Following that up a little further, that first bullet up there actually has two assumptions in it that I think are worth tearing apart a bit. One is the assumption on the actual levels, and the second is the underlying assumption that TDH-positive virulence factors is the determinant that we should be focusing on and that I'm not sure what the alternative is, but I think we should take a look at that basic assumption first and then look at whether or not the values are reflected of what's actually occurred.

DR. POTTER: Okay. Hold that thought.

Andy, your comment is about--

DR. DePAOLA: Well, using the values that are up here, what you'll find is that in a typical meal you'll end up with ten to the two to ten to the three pathogenic Vibrio parahaemolyticus and perhaps even higher if they go through some growth after harvest, and as you look at almost any of these models as you get down around ten to the two, you're having one case in 10,000 meals; ten to the three, you're having one case in 1,000 meals; and as you look at the number of meals, I think ten to the seventh on the Gulf coast, and even with these low levels, we're predicting, 100,000, 10,000 cases at these low levels.

So I think we need to be careful about adjusting these levels of Kanagawa-positive.

DR. POTTER: Okay. Andy, let me ask one follow up question. Then I'd like to get back to Bob's point. How do those number of cases that you're projecting with these assumptions match the number of cases assumed in the recent paper from CDC?

DR. DePAOLA: Well, they're assuming or they had approximately 25 cases a year, and you multiply that, that would be 50 cases from the Gulf coast--I mean 500 cases per year from the Gulf coast, figuring the 20-time multiplier, but if you have ten to the seventh meals during the summer from the Gulf coast, and one in 10,000 is causing an illness--or let's say there's 100 per gram. You get one in 10,000. If there's--100 per meal, that's one in 10,000. That's 100 cases right there. If you have 1,000, then you have 1,000 cases, and so what it suggests is that most of the cases are coming from relatively low doses.

DR. POTTER: Okay. Thanks.

DR. EKLUND: Can I ask a question?

DR. POTTER: Mel.

DR. EKLUND: Yes, I'd like to ask a question. When you say low doses, is this based upon--

DR. DePAOLA: It's based upon what the models predict. We don't know what the doses are and the oysters that made the people sick.

DR. EKLUND: I got you.

DR. POTTER: Thanks. Now let's get back to Bob's question. Is TDH-positive as a laboratory indication of virulence adequate, or should there be a different indicator or multiple indicators? Is it acceptable to peg this on one measure, one factor? Does anybody care?

DR. ACHESON: From what I heard, the TDH-negative are not pathogenic, but what we didn't hear is whether all TDH-positive are pathogenic. That gets back to an earlier point, is it could be genetically positive but not expressing or not expressing at low levels which I guess we don't know the answer to that. What I heard is that like 95 to 97 percent of clinically relevant Vp were TDH-positive.

DR. POTTER: So is that--

DR. EKLUND: Marianne, is that figure correct?

DR. MILIOTIS: Well, not all Kp--well, animal studies have shown that not all TDH-negative strains are negative. They do produce some symptoms. As I mentioned in my first presentation, we actually have a strain from New York, from the New York outbreak, that's TDH-negative, TRH-

negative, urease-negative. It does produce fluid accumulation and diarrhea depending on the animal model we've used, and in fact Honda, I guess was the 1970s in Japan, he isolated--from an outbreak in Japan, he isolated TDH negative or what he called KP-negative strain at the time which produced diarrhea, and it formed enterotoxin.

We don't know--our question to you is 95 percent of the clinical isolates are TDH positive. Someone, I think you, asked the question of five percent, what is the significance of five percent. That may be significant. It may not.

DR. POTTER: Okay. I think for the committee's purposes what we've heard is that illness has been associated with positive and negative strains, but most of the clinical isolates, nearly all of the clinical isolates are positive, and the question is is the slack too great, is the error going to be unacceptably high for the purposes of the risk assessment to use this as your indicator of virulence. Peggy.

DR. NEILL: To answer the question about whether this is acceptable, I think at this point it is. My understanding is that the majority of strains--

DR. POTTER: Could you speak up just a little bit?

DR. NEILL: The majority of strains from clinical specimens have been TDH-positive both phenotypically as well as genotypically. The isolates that came from patients which were TDH-negative, if memory serves me correct, were from stool specimens and in which case one would then have a question of whether you simply picked the wrong colony off the plate, and it messed the TDH-positive Vp.

So to my understanding, at the moment there's still a pretty high correlation, although vis-a-vis Dr. O'Brien's point this morning, it will remain to be seen whether this particular .4 percent estimate, if it's derived on the basis of the PCR results could potentially be an overestimate.

DR. POTTER: Alison.

DR. O'BRIEN: Actually, I just wanted to add--what I was going the ask was if we assume that about 95 percent of clinical isolates are Kanagawa phenomenon-positive--however you want to call it--in five percent or not, where are the five percent coming from? Are they coming from diarrheal stools, or are they coming from blood cultures, and I think you answered the question which makes me more confident that the marker, it might be a surrogate. It may not be what's really the virulence determinant.

We haven't decided that at all, but it's associated. It's reasonable. If it's coming--if the isolates coming from the more ill patients are basically uniformly positive, then I'm quite confident. I feel much more confident.

DR. POTTER: Okay. Other comments on that point?

Okay. Are water temperature assumptions representative? Marianne, do you want to elaborate on that point?

DR. MILIOTIS: Okay. That was Marleen's slide. The water temperature is what we got from the buoys, and are they representative of the areas where the oysters are harvested from? That's the buoy data, and also because we're using that water-air differentiation that John mentioned, is that acceptable?

DR. BUCHANAN: Just a minor question. When do the oyster men go out in the morning, and when do they come back?

DR. MILIOTIS: The one person that Mark and I spoke to--

DR. POTTER: Please speak into the mike, Marianne.

DR. MILIOTIS: Oh, I'm sorry. The one person that Mark and I managed to get a hold of said they go at sunrise and come back at sunset. They go out and within an hour that they're out, they start harvesting.

DR. BUCHANAN: So the temperatures that you have that are based on daytime temperatures would be realistic for these.

DR. MILIOTIS: What we've done is we took noontime temperatures because it's from morning to late afternoon. So we took it at noon which is basically the middle of the harvest time.

DR. BUCHANAN: Okay.

DR. POTTER: Does it sound reasonable? Does it make sense? Angela.

DR. RUPLE: I'm not sure how assessable that data is, but I think that at least some of the states, as part of their monitoring program, actually measure water and air temperature when they harvest the oysters. Maybe looking at some of that data if it is available would help you to determine if what you're using is indicative of what's actually happening.

DR. MILIOTIS: Thank you.

DR. POTTER: Okay. Other comments on this point? Dane.

DR. BERNARD: Thank you, Chairman. Based on what we've seen, I don't feel that we personally have enough information presented to say one way or the other regarding this assumption, however I would think that that type of information shouldn't be that difficult to get in terms of interviewing industry for experience and/or measuring actually oyster temperature as it comes off the boat or on the boat.

DR. POTTER: Andy, do you have something to that point?

DR. DePAOLA: Yes. That's actually the plan. We plan to validate the buoy data with the data from the state shellfish programs where they collect water temperature and air temperature associated there. So we will validate that buoy temperature using that information from the states.

DR. POTTER: Okay. There are a number of other points on this slide. It's, according to my watch, eight minutes past lunch. What is the pleasure of the committee to finish this slide one point at a time? Would you like to make the few comments you're making all together, or do you want to break and restart after lunch? Anybody out there. Break. Okay. Don, parting shot?

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DR. BURR: I just have one last comment. I just want to reenforce we really had nothing to do with the oysters that are being served for lunch, but we really would appreciate if you kind of kept track of the number that you ate and whether you had the red or the blue plate.

[Laughter.]

DR. POTTER: All right. We'll go ahead and take a break. We'll want to return at one. We'll, as quickly as possible, finish this up and then get to our public comment.

[Whereupon, at 12:09 p.m., a luncheon recess was taken, to reconvene at 1:00 p.m. this same day.]

cc

A F T E R N O O N S E S S I O N

[1:20 p.m.]

CHAIRPERSON WACHSMUTH: Okay. I think we'd like to resume where you left off, Dr. Miliotis. You had a list that we were working our way down of assumptions or questions.

DR. POTTER: All right. Can we get that list back up on the screen?

DR. MILIOTIS: Before we start, I'd like--

CHAIRPERSON WACHSMUTH: You need to use the mike.

DR. MILIOTIS: Okay. I'm sorry. But before we start, I'd like to introduce two more members of our task force who might be appropriate to answer some of the questions as well. Bill Watkins is my colleague, and Mark Walderhaug is a good man. He's been mentioned a lot today as well.

DR. POTTER: All right. Marianne, it is on. Talk into it and speak loud.

DR. MILIOTIS: Like this?

DR. POTTER: That's great.

DR. MILIOTIS: All right. Our next assumption is time after harvest to refrigeration. As Andy mentioned, we used the 1996 and 1995 survey data, and for the west

coast we used information we obtained from the Pacific Coast Shellfish Growers Association. Is everyone okay with that? Are there any comments?

DR. POTTER: Okay. Comments from the committee on your comfort level with this assumption?

DR. SEWARD: I'm very comfortable with it.

DR. POTTER: Thank you, Skip. If anyone is not, please say so before we go on to the next slide.

DR. MILIOTIS: Okay. We've assumed that there's similar growth and survival between pathogenic and nonpathogenic. Does anyone have any data or any reason why we cannot assume that?

DR. POTTER: Alison?

DR. O'BRIEN: No, I have no data. I just think it's an assumption.

DR. POTTER: Okay.

DR. MILIOTIS: Is it a valid assumption?

DR. O'BRIEN: Unless proven otherwise.

DR. MILIOTIS: Is it reasonable for our model?

DR. POTTER: All right.

DR. MILIOTIS: The cooling rate, once the oysters are under refrigeration after harvest, we have assumed a uniform distribution, and as Andy said, we've

assumed a range between .5 to 10 hours. Is that feasible? Should it be longer? Should it be less time?

DR. SEWARD: I would just comment that based on work with other meat products, that seems like a very reasonable assumption to me, based on other similar-type meat products that we've measured for time to refrigeration. It seems very logical.

DR. MILIOTIS: Okay. Anyone from the seafood industry?

DR. POTTER: Mel?

DR. MILIOTIS: The shellfish people, any comments?

DR. POTTER: This is for the committee, Marianne.

DR. MILIOTIS: Okay. I'm sorry.

DR. EKLUND: I think in general this is acceptable.

DR. POTTER: Okay.

DR. MILIOTIS: Okay. We've assumed that there's equal consumption patterns between the susceptible and nonsusceptible population.

DR. POTTER: Angela.

DR. RUPLE: Just a comment on that, Marianne. As everybody is aware, there's been a huge emphasis on

education the last few years, and as Michael alluded to earlier, there's been several education campaigns and at least one study that I'm aware of that our agency funded through ISSC, they were able to show that 76 percent of the people who were not aware of the risks, once they became aware of those risks, said that they would change their eating habits and they would stop consumption of raw oysters.

So I don't know if there's enough concrete information out there to give you an appropriate difference between the consumption patterns of susceptible individuals and healthy individuals, but I think those results at least could be made available to you and maybe would be helpful. I think this is a stretch of an assumption. It may have been true before all the education campaign started, but I think that there has been some effect that might need to be considered.

DR. POTTER: Spencer.

DR. MILIOTIS: Thank you.

DR. GARRETT: Thank you. Spencer Garrett with the National Marine Fishery Service. I think that's a significant point that Angela is bringing up, and if I remember that study, it was something like 60 percent of the people who were not aware of the consequences of eating raw

oysters that did not know their medical condition. When made aware by an appropriate risk communicator, such as a physician or health care professional, would stop eating oysters, and I think it was 70-some-odd percent or 76 percent--I forget the figure. Angela would know--indicated that they would also stop eating oysters when they were made aware of it, and they in fact did consider themselves at risk.

So I think you're seeing a, if you would, changing paradigm in risk communication and the effectiveness of risk communication. So I don't know how you plug it in your model, but I would think that you could take that data and at least put some sort of factor in for that particular--you may not want to use those numbers, but I would think you could put some sort of a factorial in there for that late breaking information. Thank you.

DR. POTTER: Okay.

DR. DiNOVI: Can I comment?

DR. POTTER: Yes, please.

DR. DiNOVI: Really--this is maybe too subtle, but really the problem here is once the decision to consume has been made is when the risk begins. I'm aware of the trends and the data that education has shown. That's true. In fact, in May I reported on some information that I had

that showed perversely people with higher levels of education were more likely to eat more raw oysters even knowing the risks, which that study suggested perhaps it was an intellectual control issue going on, but the fact here is what we're worried about is will people, once they decided to eat oysters, will not affect the number that they choose to eat. I think that's more to where this specific question is going.

DR. POTTER: Okay. Spencer, do you want to respond to that?

DR. GARRETT: Yes, just to point out I certainly understand from where you're coming and understand the construct of that particular model, and also I think, as I indicated, we provided some additional data that we're now going to send to you that corresponds to your earlier conclusion that people with higher educations actually eat more oysters, but again, it just seems to me that risk assessments over time are always reassessed.

I mean that's one of the 11 cardinal rules and general principles of risk assessment. So as we proceed, I would urge you to try to figure out some way to take advantage of these changing paradigms, but I certainly understand the subtlety of what you are saying.

DR. DiNOVI: One of the things that the undating of the model will do with the changing numbers is predict fewer and fewer people should be getting sick. That is certainly true.

DR. POTTER: Later, when we're done with this section, we will go into the public comment period. There are two commenters who have signed up, Robin and Bob Collette. Will either of those comments include--reference this data set?

Okay. Fine. So perhaps we'll get some more information at that point.

DR. MILIOTIS: Okay. Next question: All virulent strains are equally virulent. In other words, all TDH-positive strains are equally virulent.

DR. POTTER: Comments on the virulence, that within species TDH-positive subset of Vibrio parahaemolyticus, are virulent strains equally virulent within the model an appropriate assumption or not? David.

DR. ACHESON: I'm not sure it's an appropriate assumption. I didn't see any data to say that it was or it was not, and I know if it's too negative, I'm going to be asked to come up with something better, and I can't. It seems like a logical way to go, but that statement, as put up there I would not necessarily agree with.

DR. POTTER: Okay. Other comments?

DR. BURR: For us, in the future, I guess I'd say we're attempting to set up some animal models for Vibrio parahaemolyticus, and I think the goal of that is to see if we can look for differences between strains and look for differences, and I think that's, I think, down the line that we'd like to do that hopefully could be put in for refining that.

DR. POTTER: David.

DR. ACHESON: I got the sense from some of the work that Andy presented that maybe some of that 03 strain that was a recent cause was maybe more virulent than some of the others, but you didn't specifically say that.

DR. DePAOLA: There is a higher attack rate with that, and it has the ability to become pandemic to cross into other continents, and some of the epidemiological data from Texas showed that 17 out of 17 people who shared a common lot of oysters all became ill. Now, that could be from two different causes, one that the organism is more virulent, in other words a lower dose could cause illness, but it could also be that the strain grows better in oysters and it reaches higher numbers than other strains. It has more of this epidemic virulence.

So it would really take some animal studies or something where you control all the conditions and give each of these strains a dose range. So I don't think we're prepared to say that that's a more virulent strain at this point.

DR. POTTER: Peggy.

DR. NEILL: I think we don't know the answer to this particular question as its phrased up on the screen. I also don't know that we might be asking the question correctly. There is a paper by Mark Tamplin from not too long ago that, as I recall, was noting the genetic heterogeneity within the population in the oyster, but then in those situations in which they actually had suspect lot paired with clinical case, there's only one of the--I think it's PFGE. There's only one PFGE type in the patient, although the oyster that they consumed contained several PFGE types.

So whether there's another feature of the organism that we're not measuring correctly that is the reason for that particular isolate in the patient to be more, quote, virulent, or whether it simply outcompeted the others, I don't think we know.

DR. DePAOLA: Well, I think the paper you're mentioning concerns Vibrio vulnificus and not Vibrio

parahaemolyticus, and you're correct. It doesn't really address that one strain is growing faster, and of course the problem with vulnificus is this we don't have a means of differentiated virulent from avirulent, whereas with the Vibrio parahaemolyticus, we're basically saying that all bugs that have the TDH gene or they're Kanagawa-positive are equally virulent.

DR. NEILL: But I thought there was some data to this point from Texas. No? For Vibrio parahaemolyticus.

DR. DePAOLA: Are you speaking about the paper by Tamplin or another paper?

DR. NEILL: There is a paper by Tamplin, you're correct, but I also thought that the parallel data existed at least more recently from Texas or related to the Texas cases.

DR. DePAOLA: The Texas Health Department presented a paper at ASM this year, and they showed that all the clinical strains from Texas had near identical pulse field type, yet the nonpathogenic strains had a variety of different pulse field types.

Now, interestingly, the 03:K6 strains from Texas are a little bit different than the ones from New York, and this is even more pronounced when you use ribotype than when you use pulse field. Now, whether one strain is

more virulent than another, yes, that is not really helpful in determining that point.

DR. POTTER: Other comments? John?

DR. KOBAYASHI: One way of looking at existing data for variations on virulence would be looking at the outbreaks that have been reported and looking at hospitalization rate, mortality rates, and other occurrences of complications. I'm not aware of a large variation in those, and frankly I haven't looked at that data. I imagine you could do that with the CDC folks.

One caution would be comparing hospitalization rates or durations of hospitalizations in other countries such as Japan. I understand that at least up until recently it was very common to have extremely long periods of hospitalizations for illnesses in general there as compared to this country, but perhaps mortality would be a good comparative measure on Japanese data versus American data.

DR. MILIOTIS: So is this a yes or a no?

DR. KOBAYASHI: Well, my comment is if you look at the various outbreaks that have been reported, and you don't see a, say, five or tenfold difference in morbidity and mortality, I think it's reasonable to assume for the purposes of this risk analysis that we're dealing with equal virulence. On the other hand, if you see a

very large fluctuation in morbidity and mortality, you might have to factor that into your calculation.

DR. MILIOTIS: Thank you. Okay.

Andy showed data that the growth rate of Vp in the broth model system is four times that in the oyster at all temperatures, taking into account that we took a 26 degrees and extrapolated from the 26. Is that an acceptable assumption?

DR. POTTER: Does anybody feel that this is not an acceptable assumption? Mel.

DR. EKLUND: Well, I think it's--are you speaking of specific strains here or mixed strains?

DR. MILIOTIS: Generally.

DR. EKLUND: I'm sorry?

DR. MILIOTIS: This is our model--for our model, we're looking at Vibrio parahaemolyticus grown in broth and grown in oysters.

DR. EKLUND: What I'm saying is it depends--like I mentioned earlier this morning, it depends on if you're looking at an individual organism or a combined group of strains because they do produce phages. They produce bacteria. So you have a tremendous variation in this.

I think Marleen Wekell mentioned this morning that she found that the nonvirulent strains were outgrowing

the one that were virulent. In another case, we've shown that it depends on the capacity of these organisms to produce these compounds in the competitive inhibition. So if you're looking at an individual strain, yes. If you're looking at a group of strains, I would say no.

DR. POTTER: Roberta.

DR. MORALES: I guess I need some clarification on what the question is that you're answering. You said you had data that showed that growth rate in broth model systems were four times greater than in oysters. So to me this is a statement of the conclusions that you've made from that data, and my question is are you using the broth model system or the oyster model system in your modelling, because if you're looking at public health risk through oyster consumption, to me it would seem that the growth in oysters in miles an hour is appropriate data to use than the broth model system.

DR. BOWERS: Perhaps I can clarify this. Andy's experiment is at 26 degrees in oysters. The Miles, et al. paper is a modelled equation, and what they observed in oysters at 26 degrees was one-fourth than what the model predicted, and our question is can we take that one fourth and extrapolate that to all other temperatures. Is that a reasonable assumption?

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DR. POTTER: Spencer.

DR. GARRETT: Do you not have broth models at our varying temperature gradients, or is it just all done at 26?

DR. DePAOLA: The broth models were done at a variety of temperatures. The problem is that the oyster data was only done at 26.

DR. GARRETT: Oh, I see. Okay. So it's the other way around.

DR. DePAOLA: It's much easier to count bugs in broth.

DR. POTTER: Okay. Mel has expressed some objections to this assumption for its use in the model. Is there other sentiment that this should not be used? Bill.

DR. SPERBER: I would support the statement that this is a good hypothesis, but you should get some supporting data in oysters at the other temperatures to confirm your hypothesis. I wouldn't just accept this carte blanche for all temperatures.

DR. POTTER: Okay.

DR. DePAOLA: Yes. I think that's a very good point.

Back to Mel's comment, the one-fourth was based on a lot of different--the natural microfilaria of an

oyster that is consisting of many different strains. Miles et al. combined different studies where different strains were used in different studies. So there is that issue there with the broth data.

DR. POTTER: Okay. Marianne.

DR. MILIOTIS: So we'll take that as a no and we'll do more research?

DR. POTTER: I guess what I heard there was to use this but to validate it. Was that the proper interpretation of what you said? I see nods.

DR. MILIOTIS: Thank you. Okay. Our next assumption was the lag time to start growing for Vp, once it has been harvested, is negligible, or we're assuming a negligible lag time in our growth model.

DR. POTTER: Okay. And if I understood your comments correctly, this assumption is based on pulling the oysters out of temperatures that would support growth so that they would not be in stationary--they would be growing already.

DR. MILIOTIS: Correct.

DR. POTTER: All right. Dane.

DR. BERNARD: Thank you. Question for the panel: What happens to the oysters themselves at harvest? Do their conditions change in terms of the pH of the meat,

anything that might affect the growth of the organisms? I mean most things when under stress their chemistry changes. I would assume that oysters are the same.

DR. DePAOLA: Well, it depends on whether the oysters are maintained alive or if they're shucked. If they are shucked, then that usually results in their death very shortly, and they go through a glycolysis, and the glycogen is converted to lactic acid, and there's a fairly sharp pH drop, whereas if the oysters are stored live in-shell, there's a pH drop that is more gradual. It goes from around 6-8 to 6-2 over the course of 10 to 20 hours out of the water, and each of these pHs will support Vibrio parahaemolyticus growth, as we've shown in our growth studies which the oysters were left out and live oysters that were subjected to these pH changes.

So I guess the point is that the data was collected with animals with no control over their pH. So it was doing what it was going to do under typical industry practices.

DR. POTTER: Okay. Mel, you had a comment?

DR. EKLUND: Yes. Going back to the previous slide there, if you would, I think it depends a lot--we were talking with some of the oyster growers during the break, during the lunch here, and there's a question you had

earlier about the physiology of the oyster, and we know that during the spring and fall, I guess on the east coast the oysters spawn, and in the summertime on the west coast they spawn, and we also know that we have some cases where oysters become more susceptible to diseases, and if they're more susceptible to diseases, they're also more susceptible to Vibrio parahaemolyticus.

But I think the thing that concerns me more than anything else is that there probably is that variation. We know that in animals or oysters or fish is that when they go through a spawning, their whole physiology changes. Their protein changes. Their water changes and so on. What role does this play in the growth of the Vibrio in this environment?

DR. DePAOLA: We haven't controlled experiments to determine those factors, however these experiments at 26 degrees were repeated monthly throughout the year including their spawning times, and we did not see any months, particularly the months May, June, and July where there was any more rapid increase of total Vibrio parahaemolyticus numbers in the indigenous population than we did at other times during the warm time of the year.

DR. POTTER: Mel, does that answer your question?

DR. EKLUND: Yes.

DR. POTTER: Okay. So we stay with the earlier decision to use this but to try to validate for other temperatures. Okay.

All right. The business of lag time, Dane, you had commented on that. Is there any follow-up questions?

DR. MILIOTIS: Okay. Depuration, effectiveness of depuration. We had--as I mentioned in my concluding remarks, we looked at three--there were three studies. Two were naturally-infected oysters, and one was with lab-infected oysters, and we assumed that we would go with the naturally-infected oyster studies; and I think Bob Buchanan suggested or wanted to know why we weren't going to take the lab. This is an assumption, but this is also a question for you: What do you suggest we do?

DR. POTTER: David.

DR. ACHESON: Take the naturally-infected ones.

DR. MILIOTIS: Thank you.

DR. POTTER: Okay. Does anybody take issue with that recommendation?

DR. EKLUND: I was talking to Dr. Nacamara from Japan during the lunch hour, and I think also in Japan

they have shown that depuration is not that effective. So I think that would be a good assumption.

DR. MILIOTIS: So is it okay to exclude depuration from our model? I mean exclude the lab-infected. Correct? So you'll just be dealing with the naturally-infected oysters in depuration. Thank you.

DR. POTTER: Okay.

DR. MILIOTIS: Okay. The question arose of the consumption differences. We've assumed that consumption will be the same in all regions. Is that correct?

DR. POTTER: Let me ask a follow-on question to be considered during this. What was the earlier decision on consumption across different risk categories? We had a question about whether people at higher risk of adverse consequences of infection consumed at the same rate.

DR. WALDERHAUG: We had decided that we would get more data and incorporate that into the model.

DR. POTTER: Okay. So that's going to be modelled in, the difference. So aside from that risk-based difference in consumption, is there also a regional difference in consumption in addition to that? Okay. Will either of our two public commenters--no? No. So the committee will not be supplemented on this. So, you know,

if the committee has some sense of how the data or what the data are on this--Ken.

MR. MOORE: I haven't signed up, but I'm going to make a comment about the data if I can get an opportunity.

DR. POTTER: Oh, okay. So perhaps we will get some help there. But still, if the committee has seen any information on this or has any comment on this from other data sources--Roberta.

DR. MORALES: I wonder if maybe a simple first start at looking at whether or not there are regional consumption differences is to look at some of the USDA economic research service data and whether or not they might have some of that information available there. They usually collect that data either by state or by region, and that may be a very simple first at determining whether or not substantial differences do exist.

DR. POTTER: Do they have consumption data, or are they using either production or disappearance data?

DR. MORALES: I'm not sure because I don't know this data really well, but for many, many different food products, they do have both consumption and production. So it's either ERS or NAS that has--that would have that.

DR. POTTER: Spencer.

DR. GARRETT: Yes, thank you. As soon as I get back, we did a rather comprehensive review of the entire oyster industry many years ago, and it seems to me that there was some consumption data in there that may not be used. So as soon as we get back, we'll take a look at that and just provide it.

DR. POTTER: Okay. Thank you.

DR. DiNOVI: It's not entirely clear to me what this means. If we're going to be doing this risk on a per meal basis, the point that John made this morning was that the risk relates to where the oyster comes from more than where it's eaten, and so I'm in the exactly sure. It's very clear that there are higher consumption levels among people who live near the shore than live inland.

You know, there are not many eaten in Montana compared to Florida, but again it's back to what I said earlier. Once you've chosen to eat an oyster or have an oyster meal, is it at that point that the risk goes from zero to some positive number, and then is it simply based on where the oyster was harvested and how it was handled? I'm not sure that I understand what we would factor in exactly.

DR. POTTER: Okay. Mike?

DR. ROBACH: I agree with that, but given the fact that you've got a higher percentage of pathogenic

Vibrio on the west coast, I guess the question becomes, one, are most west coast oysters consumed on the west coast or are they shipped across country. Yes, to me the regional consumption differences fall in line with where the oyster was produced and what are the distribution practices and consumption patterns. I mean do most people eat locally-produced oysters or not?

DR. DiNOVI: Well, I don't have the numbers with me here that I looked at, but the crude answer is yes, they do tend to be eaten closer, and certainly, you'll hear comments on that, but again, I go to the question, for example: Someone in Florida on vacation that eats an oyster, are they more or less likely to become sick? I don't know that their history or their normal consumption pattern matters. It's just they're eating that oyster.

DR. ROBACH: It depends at that point in time where they eat it or, more importantly, where was the oyster from that they consumed at that point in time.

DR. DiNOVI: I would certainly agree that if there's a postharvest transport difference in growth, that would definitely matter and would have to be taken into account because that would change the number of when you consumed the oysters. So that would definitely have to be taken into account, but I don't know that--I thought I heard

this morning--I think it was Andy. Did you say this morning that there wasn't much difference on transport?

DR. DePAOLA: Yes. I think that you're saying the same thing. It doesn't matter whether--it matters whether the oyster was harvested in Washington in July. Whether you eat that oyster in Washington or Montana or New York, your risk is going to be the same no matter which state you eat it in.

Now, the retail study has shown that there's a slightly longer period of time from harvest to consumption if the oyster is shipped out of state, but that's not going to affect the model, just a day or so. I think we got .07 logs per day. So we can certainly take that retail data and extrapolate that into the oysters that are consumed inland, but I'm not sure that we have good data about how many oysters are consumed in the Midwest, and so I think it really comes down to it's the most important factor is where the oyster was harvested from, and the model will simulate how it was treated.

DR. POTTER: Peggy.

DR. NEILL: It occurs to me, as we've been talking here, that are we meaning two different interpretations of regional consumption differences because one is certainly what we've just been talking about in terms

of the physical location of harvest versus the physical location of the oyster being consumed, but another aspect I think I have and a question from this morning is whether there's data on differences in patterns of consumption, in other words the numbers of oysters eaten not just percentage of the population that eats oysters but the amount that they're going the eat at a meal, because I would begin to think that could play a role in some of outcomes in the model.

DR. DiNOVI: That is correct. Yes, that would. The data that I showed this morning was just from the Florida survey, but as Spencer mentioned, there's Maryland data that will be made available that we will take into account. It still all comes down to the distribution and how you want to look at the distribution of number of oysters eaten per meal and how far you want to break it down.

Certainly, if you're predicting number of illnesses in regions, that matters a lot because the inland states will obviously have much lower numbers than the coastal states, but if you're doing it on a per meal basis nationwide, it would not matter then. Just the number of oysters at a meal would matter, and the more distribution data you have, the better. You just have less uncertainty.

DR. POTTER: Okay. Let's go to the next.

DR. MILIOTIS: We've assumed from the CDC data that seven percent of the population has underlying conditions. Is than an okay assumption?

DR. POTTER: Peggy.

DR. NEILL: As I've been thinking about that, I wonder if I could ask you to try to expand that. I know what I think that number means, and if it does, then I don't think it's going to be valid here. Where did the figure come from? Is this the global figure of estimating that seven percent of the U.S. population has characteristics predisposing it to an increased risk of any infectious disease? Is that what this figure represents?

DR. POTTER: I think that figure, Peggy, is around 20 percent. So I think that this is a subset of that.

DR. NEILL: Then what's in the subset?

DR. BOWERS: We're taking the seven percent from a tabulation of prevalence of illness in the U.S. population which was tabulated for Vibrio, and it relates to disease conditions, prevalence of disease conditions which caused iron imbalance in the blood.

DR. POTTER: Which could include liver storage, hemochromatosis, and other underlying liver disease.

Peggy, would you like to probe that?

DR. NEILL: Yes, because then what you're saying is that seven percent of the U.S. population has an iron overload state, dialysis patients, those recurrently transfused, liver disease, hemochromatosis. Is that what's captured in here? Because if so, to my knowledge that would only predispose the persons affected with Vibrio parahaemolyticus to the more severe forms of infection such as bacteremia.

DR. BURR: I think that, yes, the idea is to get more of a handle of the slide of the CDC, and Mary can say something about it. There was a lot more. I mean, they talked about malignancy. They talked about a lot of different underlying conditions. So it may actually--what we're looking for is more of a general number that covers more than just that.

DR. POTTER: Okay. Perhaps it would be worth figuring out what that is, whether the seven percent is at increased risk of infection or increased risk of serious illness given infection, and that's something that you can do just by reviewing what the characteristics were.

DR. BOWERS: We're thinking that the--there is increased risk of infection by looking at the CDC data, because for the sporadic cases in the Gulf coast, you see 100--you tabulate 107 sporadic cases, and we didn't get the list of exactly what the conditions are. We didn't have a data base for 107, but just a yes or no on a underlying condition, and these people represent the cases. So that tells you something.

DR. POTTER: Alison.

DR. O'BRIEN: Following through your logic, it still doesn't say they have an increased risk of infection. It says they have an increased risk of clinical disease. I mean there are a lot of us that could be infected and not have disease.

DR. POTTER: Peggy.

DR. NEILL: I think what we're trying to say is that in general terms there's something about this that doesn't quite hit what we're after, particularly in terms of, probably, dissecting the difference between severity of outcome versus simply infection. There is certainly the consideration that sounds like a red flag to me, that if the only information is that something checked off the box saying underlying condition, well, what is that? I mean is a person who is on an H2 blocker the same as somebody who

has got hemochromatosis? I wouldn't want to categorize them that way up front. I'd probably try to break it down, etc.

I think another problem here is certainly that at least at the clinical grass roots level most people would feel that Vibrio parahaemolyticus is amply capable of making lots of normal people sick if they're not that sick or they're perceived to be normal. Their physicians may not obtain stool cultures, and hence they're never going to show up on a surveillance effort. So your case count is going to be overloaded towards those who got through the system, got diagnosed, got a form filled out and something checked off the box because they had a more severe illness and risk factor, but they're not really representative of the population that got infected.

DR. POTTER: Dane.

DR. BOWERS: That's a good point.

DR. BERNARD: Thank you. I had questioned that earlier myself as to what the seven percent means. We've heard that, quote, unquote, those underlying conditions are overrepresented in the sporadic cases. It could be that those with underlying conditions are overrepresented in those that consume oysters in the first place. That was my point. Because what you've done is taken a figure that represent it is entire population.

We've already seen that result related to the education levels. Is there something else in that population that represents those who would normally eat oysters?

DR. POTTER: John Kuenberg.

DR. KUENBERG: Well, my flag went up and down a couple of times because there were points I was going the bring up, but then Angela--I guess it goes to the fundamental question on that point on the seven percent. I guess the question I would ask is is there a significant reason that you need to have a percentage basis on this particular organism, this particular food to even consider in your assessment. What's the necessity of having a percentage of seven or anything else? Is there an underlying illness that's obviously evident that you need to put into the assessment?

DR. POTTER: Okay. If I understand that, because I think it does fall on something that Peggy said, is that 100 percent of the population is susceptible to infection and illness at some level. There's some subset that may be more susceptible to severe disease. Are you two saying the same thing?

DR. KUENBERG: I guess the question is would it be more useful if the assumption was revised.

DR. POTTER: Okay. So perhaps a revision or a refinement of the assumption. Spencer.

DR. GARRETT: I'm not going to address that. I was merely going to ask a clarification question. When you put up a percentage like that, do you strike out any who are known not oyster eaters like children under the age of two, for example? You know, pick an age. Or do you strike out people over the age of 93? I'm just merely asking the question. Or do you just go with the straight percentage?

It would seem to me that if you're going to have a percentage, whether it's seven percent or some higher percentage of the total population, then I think you need to try to refine it more to the best that you can, obviously, of the actual oyster consuming public.

DR. POTTER: Okay. I think I hear an endorsement here to refine and revise the assumption.

Okay. Next. I think we discussed this before lunch.

DR. MILIOTIS: Yes, this was discussed.

DR. POTTER: Okay. And this was discussed.

DR. MILIOTIS: This was discussed, and I think that's it. Thank you.

DR. POTTER: And that's it.

Okay. General comments from the committee? Earl.

DR. LONG: Earl Long, CDC. This may be peripheral, but from my very limited knowledge or familiarity with oyster eating, there was one event that seems universal in all of them, and that was consumption of alcohol. Does that have any effect on pathogenicity or infectivity?

DR. POTTER: I think that goes into, perhaps, the food matrix part of this. Are you modelling alcohol concentration into the food matrix effect? I don't know whether data exists that would let us portion out how many oysters are consumed by sober people versus people who are drinking. You know, I suspect that there's proportions in both directions, and in the absence of data, how would you suggest that that be modelled?

DR. LONG: I don't have the answer. It's just something that kept recurring.

DR. POTTER: Okay. Andy, do you want to--

DR. DePAOLA: I think it's 12 oysters per beer.

[Laughter.]

DR. POTTER: Roberta.

DR. MORALES: Actually, oddly enough, I think there may be some data on this. In North Carolina, sometime in the mid-nineties, there was an outbreak. I think it was vulnificus, and it was at one of the oyster bashes, which apparently happens fairly frequently in North Carolina, where they serve up bushels of oysters and lots of alcohol and nothing else, and there was actually a workup that was done. The average consumption at that oyster bash was 70 oysters per person, and in looking at the odds ratios for risk factors, the North Carolina Department of Public Health found that alcohol was, interesting enough, protecting individuals in terms of the severity of the illness that was manifested.

I believe some of that was written up. Kathy Kirkland was the EIS officer that did this investigation, and they did a fairly extensive survey on how people consumed the oysters as well.

DR. POTTER: Yes. I think there have been other outbreaks that were investigated in trying to look at a dose-response relationship of the alcohol consumed in alcohol units, meaning that small amounts of whiskey equaled large amounts of wine equaled large amounts of beer, but I don't know if it got at the question of or how well it

addresses the proportion, but perhaps that's something that could go into the food matrix assumptions.

DR. BURR: I'm not sure if it will pass the Human Use Committee up in Maryland, but perhaps when they do the feeding trials there we could make some suggestion what they wash it down with it.

DR. POTTER: It may increase the number of volunteers.

[Laughter.]

DR. POTTER: Okay. Comments from Marianne to the committee? Any additional help that committee members could present in writing that has not been covered here?

DR. MILIOTIS: That's an excellent, idea. Thanks, Morris. Any comments you have in writing, we'd appreciate it, and another thing which I did not mention earlier is I think our next--our final version of the risk assessment is supposed to be in November. So if we could have your comments at your earliest convenience, we'd really appreciate it.

Thank you, and thank you for your attention.

DR. POTTER: Dane.

DR. BERNARD: When you say final, what does this mean, and is it a final report or a final draft? Are you ready to do the risk assessment at that point?

DR. MILIOTIS: Someone once mentioned a risk assessment is never final, but our--

DR. BERNARD: I stand corrected, however--

DR. MILIOTIS: Our model will be finalized.

DR. POTTER: Mel.

DR. EKLUND: I'd like to congratulate the participants of the panel this morning. I think they did an excellent job in the presentation and answering questions. So congratulations.

DR. POTTER: Okay. Thanks, Mel. I think that that goes for all of us here. It was very impressive.

PUBLIC COMMENT

DR. POTTER: We now go into the public comment period. We now have three people who have signed up to comment. There's a podium and a mike there. Robin, you're on first.

ROBIN FROM AUDIENCE: First of all, I would like to thank this task force that really has worked very hard on a difficult issue, and I appreciate particularly the efforts that you have made in trying to collect the proper data, especially from the west coast. As you remember, in Chicago I was concerned about the lack of west coast data. So I do appreciate the efforts that you have made to incorporate that data.

That said, I think there is still some clarification that does need to take place. I will certainly follow up with yet another memo, which I'm sure you will all look forward to. This is clearly a work in progress. We clearly still don't know enough. We have still have, I think, more questions than we've got answers, and I think that is pretty much well recognized by everybody. So I do urge everybody that is in the audience to keep that in mind as we work through this process.

One of my concerns continues to be the use of the TDH-positive as our indicator. According to some data that I have received from the FDA on the west coast, there are TDH-positive strains out there that are being found in our waters, but there have been no illnesses associated with those bodies of water. So I believe, Marianne, that information was sent to you by Chuck Kaysner. So that is just an alert and a call, I think, for more information to be gathered. More science does need to take place.

Another note I'd like to make is that reporting from state to state varies quite widely, and I think fortunately or unfortunately since Washington has done such a sterling job of providing data that we actually be penalizing ourselves at least in Washington State on the

west coast because of that data where it appears to be maybe more illnesses on a percentage basis.

I also would caution you in adopting a model that's based upon insufficient data. I think some of the numbers that we heard earlier were of concern to us in terms of illnesses in the population related to eating oysters, and I also am concerned about postharvest treatments were even mentioned in the context of this risk assessment, because that belongs, I think, more rightly under control and management later on down the road. I don't really see where that fits into a risk assessment of raw oysters.

And I'd just like to conclude by saying I think we can draw one conclusion at least in terms of a link between the trend of more educated, more high income people eating oysters, and that clearly is that if you eat oysters, you will be richer and you will more intelligent. Thank you.

DR. POTTER: Thanks, Robin. I'm properly motivated. I can certainly use both of those. Bob Collette.

MR. COLLETTE: I know it's late. I'm going to keep my comments brief. I am Bob Collette, representing the Molluscan Shellfish Institute. I want to thank the chair

and the panel and the committee for a few moments to share a few thoughts.

As Dr. Bowers was talking and discussing and introducing the concept of Monte Carlo, an image came to mind, and I thought isn't that someplace near the south of France where there's a lot of gambling taking place where the probability of the outcome of that gambling is not very well known, and I wonder if that had anything to do with this process this afternoon. That was supposed to be the amusing part of my comment.

With respect to MSI's view of what we've heard today, to the degree that the risk assessment helps to identify, helps FDA and helps the industry and state regulators identify areas where we need to do more work with Vibrio parahaemolyticus, I think that is a process that the industry supports and is very interested in.

I was little bit disappointed, although I certainly understand under the circumstances, that the committee was discouraged from focusing a little bit more on the research--identification of research priorities associated with the risk assessment process. I think that given the time, I understand that, but I would encourage or urge the committee members in your follow-up comments to the task force to maybe spend a little more time in that area,

because what I see here, and those of you sitting at the table are better qualified to make these judgments than I, but what I see here is a risk assessment that has a great deal of uncertainty in it.

And that leads me to my next point, and that next point is I also very strongly believe that when we leave this room, we have to be careful how that's communicated, that there is a great deal of uncertainty here as far as I can see, particularly with respect to the dose-response model. There's a lot of questions as to whether you've got the right model and the information that's being fed into that model. There's not a lot of data points there as far as I can see.

So I think that those things need to be brought in mind. I realize that there are limitations, and we have to live with those, but I think it's very important that the FDA and all those involved in this process walk away from the table here understanding that when we communicate the results of this risk assessment, that we make it very transparent as to the uncertainty of what's been done here.

With respect to the assumption on growth, I just wanted to make a quick comment about that as to whether the virulent organisms are going to behave the same way as

the entire population of Vibrio parahaemolyticus. One thought--and I don't know if this has any validity whatsoever, but I wondered if there are any analogous situations where that relationship is understood with other organisms and whether that's something that could be looked at.

And I guess the last thing I'd like to encourage is that, again with respect to the communication, I think it's been said here that this is--I think yet--actually I was here at the Listeria risk assessment, and it was made mention of the fact that there's a cart before the horse type of the situation here. You need to go through this process in order to find out what you don't know. So I would like to also caution that when we communicate about what's being done here, that in addition to being transparent about the uncertainties, that we also indicate and also bear in mind that this is an ongoing process, and that sort of mimics what Robin also said.

With that, I just want the thank you for the time.

DR. POTTER: Thanks, Bob. Ken.

MR. MOORE: I'm Ken Moore representing the Interstate Shellfish Sanitation Commerce, and I, too, would

like to thank the group for giving me an opportunity to make a couple of comments.

First, I understand that microbial risk assessment is a new science, and I certainly appreciate your efforts here today in trying to advance that science, but I've watched the level of apprehension and uncertainty rise since eight o'clock this morning. That does give me some concern. I think it's important that we all be judicious as we move forward. I think we have to recognize that our actions collectively, the actions by this body, the actions by FDA, actions by ISSC certainly can impact a lot of people, particularly in how we use the information.

So I just ask that we all remember that as we move forward, and lastly, I'll comment on the regional consumption differences, and I think, too, we're talking about distribution patterns of shellfish across the United States. We presently have the Research Triangle Institute from Raleigh, North Carolina under contract conducting an economic assessment of postharvest treatment of shellfish. We expect at least a draft report sometime next month, and I do expect that they will be addressing issues of regional consumption differences in distribution patterns, and as soon as we get any information, we'd be glad to share it with you.

And again, thank you very much.

DR. POTTER: Thanks, Ken. We'd greatly appreciate those new data.

COMMITTEE DISCUSSION

DR. POTTER: Final comments from the committee? John.

DR. KUENBERG: Just a thought in passing, a historical note relative to some of the information that's gone on including time temperature decisions and grids.

A number of years ago the National Advisory Committee did involve itself in shellfish issues, and that fine idea came from Dr. Bill Sperber at one point in time. So we've had prior association with this, and I'm glad to see that, and although it had been mentioned earlier, I'd like to acknowledge prior work on the issue.

DR. POTTER: Thanks, John.

All right. With that, the panel is free to escape, and we will see what the committee pleasure is on draft document on bare-hand contact. You should all have in front of you the comments that were agreed upon yet, the day before yesterday, whenever it was we dealt with this.

Peggy has very nicely tried to restate some of the things to improve the clarity and focus on some of our composite attempts. LeeAnne is going to get her machine

going up there, and we'll get this redraft on the screen, and I'd like to stage this discussion.

The first question, I guess, is whether the committee has--sees a great need to severely and critically edit and re-discuss some of these issues, in which case we may want to defer, send out paper copies and get comments back on paper to Cathy. If the committee feels, however, that we're pretty close here and that this is going to be a fairly simple and straightforward editing task that can be expeditiously carried out by overworked and fatigued committee members right now, we can go ahead and nail it, approve it, and be done with this issue.

So to address the first question, is this something that you want to try to work through in the next half hour or so, or do you think it's going the require a great deal more effort than that? Bill.

DR. SVEUM: I'd be willing to work through this in the next 10 minutes.

DR. POTTER: Okay.

MS. JACKSON: I was voting for two minutes.

DR. LANG: I support that as long as we keep our focus on content and not style.

DR. POTTER: Okay. Content and not style, is that agreeable? For those of us who never had style, that will not be hard to achieve.

Okay. Well, let's have a go at it. If things break down, and people start getting nasty and belligerent, well, we'll punt.

Okay. The first paragraph--what have you done?

MS. JACKSON: I'm making it bigger so everything can read it.

DR. POTTER: Okay. Isn't technology great?

Okay. The first paragraph, any comments? The insertion here in parentheses in bold print is put there so that thereafter in the document instead of the acronym it says "the committee". Do people first accept the change? I see nods. All right. Is the rest of the paragraph still acceptable to the committee? Okay.

Paragraph two, no changes. Still okay? Okay.

Paragraph three, a change to explain what extent the total exposure time means. This prevents not only transmission to the public but also other employees, if infected, further extends the chain of transmission. Okay. David.

DR. ACHESON: To something that's not involved, just to raise the question here, the implication is that if you've got a worker, food worker that's in an establishment who is having diarrhea every half hour. As long as he's not in a food contact surface, it's okay for him to remain there?

DR. POTTER: Perhaps Skip could say what the Americans with Disabilities Act says about that.

MR. SEWARD: Yes. I mean, I think if they're not working in a food contact area or a food preparation area, you know, they should be allowed to work. I mean, if it's outside cleaning the outside of the lot or things that--you know, washing windows in the front of the restaurant and things like that, those--you know, I think you have to allow them to do that.

DR. ACHESON: The concern of transmission of food-borne pathogens in the bathrooms that he or she is using along with the other employees, that's not of concern?

DR. SEWARD: Well, I would--you know, one of the arguments that we've always had is that when you have a quick-service restaurant or any restaurant, you have literally hundreds of customers coming in every day who may have diarrhea or other diseases, and they're not washing their hands, and they're touching the faucets and so forth.

So, to me, that's a much bigger problem facing restaurants in bathrooms than your own employees, is what's happening with just your customers.

But you know, they have to take care of themselves, and they have to--you know, if they're sick and they're having diarrhea every half hour, they're probably not going to stick around and be at work, and you wouldn't know that unless they tell you or something anyway. So, I mean, it's not just a simple matter of excluding them in a written document.

DR. POTTER: Peggy.

DR. NEILL: Perhaps the chair could clarify that items in this document pertain only to the matter as referenced in the title recommendation on bare-hand contact with ready-to-eat foods.

DR. POTTER: Okay. So, in other words, this document doesn't address whether they should be in the parking lot.

DR. ACHESON: That's fine.

DR. BERNARD: That was my take.

DR. POTTER: Okay.

DR. BERNARD: Just silence on it doesn't constitute endorsement, but I guess Skip's taking out all the bathrooms in McDonald's.

DR. POTTER: Maybe you could leave the bathrooms in but take out all the sinks so there are no faucet handles for them to touch.

DR. BERNARD: Where's the guy from Sloan Valve Company when you need him?

DR. POTTER: Okay. The next paragraph, there were more extensive edits, and it was felt easier to just retype it rather than insert the edits. Peggy.

DR. NEILL: I believe there is an inadvertent loss of the last sentence of the stricken paragraph from my attempted rewrite, and I would wish to see it included. It's the sentence about hand-washing helps control cross-contamination from other sources.

DR. POTTER: So you're processing the new paragraph, but the addition of the last sentence from the original paragraph.

DR. NEILL: It was the intent. I think I may have forgotten to actually rewrite it out.

DR. POTTER: Okay. Mel.

DR. EKLUND: I think it's the third sentence there. It says proper hand-washing. Mike and I talked about this yesterday or the other day, and we were kind of wondering about personal hygiene which would cover some of

the same things, and I don't know if it's necessary or not, but I just thought I'd bring it up.

DR. POTTER: Sense of the committee? Do you want to put personal hygiene in there after hand-washing?

DR. BERNARD: The title of document is recommendations on bare-hand contact with ready to eat foods. It's not a do-all, be-all of disease prevention.

DR. POTTER: Okay. So you think this falls into the same category as the earlier concerns. Mel.

DR. EKLUND: Well, I think if you don't have good personal hygiene, it can definitely go to your hands.

DR. POTTER: Okay.

DR. EKLUND: I don't know. It's up to the committee. It was just a thought that Mike and I had.

DR. POTTER: Peggy.

DR. NEILL: I'm just wondering about what the intent is. I think maybe Dane and I have the same sense of not being quite sure what you're after with the phraseology of personal hygiene.

DR. EKLUND: Well, just one example is when you go to the bathroom, you use proper--you know, things like that. Those were the things we were talking about, and it's--you know, we're kind of iffy on it. I just thought I'd bring it up since Mike had talked about it too.

DR. POTTER: John?

DR. KUENBERG: It does go, perhaps, beyond the scope of the charge, but the code itself goes basically into personally cleanliness to include hands, arms, fingernails, jewelry, and outer clothing. So I don't know if it goes beyond the charge or not of what you were saying, but personal cleanliness is the term in the code that deals in addition to hand-washing.

DR. POTTER: Dane.

DR. BERNARD: As a proposed fix, in the paragraph, the line next to the bottom: "essential and integral component of a strategy such as that outlined", and reference that part of the food code that John just mentioned. You've wrapped it all in there without burdening the document beyond what it's intended to do.

DR. KUENBERG: Okay. Just incorporate Section 2-3, personal cleanliness, if you choose to do that.

DR. POTTER: Okay. You said put it in after strategy as a parenthetical expression, component of the strategy such as that outlined in--

DR. KUENBERG: 2-3, Two, dash, three.

DR. POTTER: Two, dash, three, 1999 Food Code.

DR. KUENBERG: Parentheses--if I may, "parentheses, personal cleanliness", so the reader understands.

DR. POTTER: Okay. How does the committee feel about that? Do you want to keep the parentheses in or exclude?

DR. SEWARD: It's fine.

DR. POTTER: Okay. It's fine. So we're up to--at least up to the level of ambivalence. We'll go with that. Ambivalence is fine.

Okay. The next paragraph.

MS. JACKSON: So are we keeping the paragraph and deleting the other one?

DR. POTTER: We're deleting the original paragraph, keeping the new paragraph.

Okay. Looking first at the new language, the stuff that's been stricken and the words added. Peggy.

DR. NEILL: Katie Swanson left her comments, and it's a tiny thing, but in the first sentence, LeeAnne, where it will read "the committee concludes that minimizing bare-hand contact", changing "of" to "with".

DR. POTTER: Okay. Anybody not in favor of accepting the edits as they exist on the screen? Mike.

DR. JAHNCKE: "The committee." Right?

DR. POTTER: Yes.

DR. JAHNCKE: Previously, when we referenced the food code, as far as personal hygiene, if we look at this paragraph now, we talk about restriction, exclusion, restriction of ill food workers and proper hand-washing. Should you reference again the section in the food code or not? I mean, we did previously, and now on this paragraph we seem to leave it off.

DR. POTTER: John.

DR. KUENBERG: The potential fix is "personal cleanliness". So if you want to use it, you could insert those words. Perhaps I would propose, if we're going to mess with this, "exclusion or restriction of ill food workers, personal cleanliness and proper hand-washing". Would that work?

MS. JACKSON: Would you say that again?

DR. KUENBERG: "Personal cleanliness" inserted after the word--put a "comma, personal cleanliness" after the term--I've lost it. After ill food workers and before the and. "Exclusion restriction of ill food workers, comma, personal cleanliness and proper hand-washing."

DR. POTTER: The proposed fix puts in the words that we left out of the paragraph above by putting in a reference to the Food Code. What's the pleasure of the

committee? I think there are three options: one, leave it out; two accept John's wording; or three, reference the same section of the code. Any preference? Bill?

DR. SVEUM: I kind of want to leave it out. We have included it in the previous paragraph and about half of our committee members are no longer here, and a couple of days ago we put together this whole document after a lot of thought, and discussion centered on three principles: exclusion of ill workers, hand-washing, and then intervention such as gloves or utensils. So I think we're skating into some new territory here by making too much of personal hygiene.

DR. POTTER: Okay. Mike has withdrawn that. So we're back to accepting the edit in this paragraph or leaving it as it was.

DR. SEWARD: I'd like to accept it with the changes.

DR. POTTER: Okay. Does anybody not agree with Skip? All right.

The next paragraph. Do we want to add the words "interrelated" or "interlocking" to modify the word "intervention" and change the word "strategies" to "steps"?

DR. SEWARD: I guess from my standpoint I don't see that there's necessary. They're extra words, but

I think it's pretty clear, the way it was, what the intent was and what was meant.

DR. KUENBERG: I agree with Skip.

DR. POTTER: Okay. So we'll delete the changes there.

Peggy, do you want to defend?

DR. NEILL: I put in the word "interrelated" or alternative choice of "interlocking" because I felt it was not clear and because we had had discussion of whether we were inadvertently creating a document that purveyed a menu, a choice, and so that was why I chose to capture that point in a manner that I thought was a little more forceful, that these are interrelated intervention steps but without necessarily implying a particular sequential aspect.

DR. POTTER: Okay. So the intent of the change was to make certain that it was clear that it was all three, not any one of the three. Dane?

DR. BERNARD: I think, Mr. Chairman, you just said the magic word. The intent was all three, and if we just change the word the in front of "three" to "all", I think you've got it.

DR. POTTER: Stephanie.

DR. DOORES: I was just going to suggest that--I concur with Dane, but I was going to suggest that the

possibility of using the terms "interrelated" or "interlocking" sounds almost like you could used gloved hands or utensils without having washed your hands, and I don't think that was our original intent, but I think "interrelated" might suggest that you could go that way, when I don't think that was the way that was intended. I think we intended always to have washed hands and then go a step further. So I would suggest that I concur with Dane about all three.

DR. POTTER: Peggy, does that capture your thought?

DR. NEILL: Yes.

DR. POTTER: Okay. So we say "implementation of all three intervention strategies". Steps. Okay. The committee wants "steps".

DR. SEWARD: Well, "steps" sounds like a progression more than "strategies". I think "strategies" sounds more like what we were after. So they're not three steps.

DR. NEILL: Why not just change it to "interventions"?

DR. POTTER: "Implementation of all three interventions." Okay.

DR. NEILL: I work for Madeline Albright on the side.

DR. POTTER: Okay. "Implementation of all three interventions outlined above" is how we go. Okay.

Next, the committee noted that additional research is needed on the benefits disadvantages, and public health outcomes of bare-hand contact with ready-to-eat foods. Sold.

All right. We accept the edits. We're done. Thank you very much. This will be typed out and clean copy provided to all of the members and will go from the committee to the agencies. Thank you.

DR. DOORES: Did we get that in 10 minutes?

DR. POTTER: What?

DR. DOORES: Did we do that within 10 minutes?

DR. POTTER: Close, yes. We met Bill's goal.

And we'll now have a break until three and come back for the small plant hazard analysis guidelines.

[Recess.]

UPDATE ON SMALL PLANT HAZARD ANALYSIS GUIDELINES

DR. POTTER: Will the remaining members of the Royal Order of the National Advisory Committee come back to order and we can get done with this meeting?

cc

The next thing up is an update on the small plant hazard analysis guidelines. As you recall during our last meeting, FSIS presented the guidelines for the committee's input and received that input and has modified the guide and then will now re-present it and how the agency intends to use it.

DR. ENGLEJOHN: Thank you, Morris.

First, as the acting chairperson of the Meat and Poultry Subcommittee, I want to thank the subcommittee members in particular for taking the time to continually work on the drafts that I've sent since the May meeting, of which there were several drafts, as well as the full committee because I did get substantive comments from the full committee since the last meeting in Chicago.

We have made several changes to the document. In particular, we did identify the document as an FSIS document developed with consultation with the committee, and FSIS did go through the document to make sure that it did not conflict with regulatory requirements we had related to HAACP.

Although it didn't contain some of the stuff we would have liked to have seen related to Listeria in particular, we still felt that the document was an excellent source of guidance for the small plants and felt that it

would be timely to make it available. We had a public meeting in August for which we had intended to make it available. We also have a packet of information of more broad issues for the very small plants that was being printed and is being made available to the very small plants, and so it was the agency's decision that it was timely to be able to take this draft document and rename it and identify it as an FSIS document and then plan to make that available to all the very small plants. So that would be roughly 6,000 facilities around the country.

I do want to say that I have received some verbal comments since this document has gone out, and then today I did receive the first of the written comments from industry and in particular from the American Association of Meat Purveyors, of which they made some comments I'll share briefly with you in that it's their opinion that the document is still too technical, that it doesn't address chemical or physical hazards, and that it doesn't fully explain what to do with the information, and then, finally, it's too narrow in that it only addresses a few of the meat products as opposed to the broad range of products.

These are the same issues that the full committee brought to the subcommittee. We do recognize that, however, with implementation coming about in January,

we still feel that the document has some excellent information that should be shared at this time, and so with that, it would be my recommendation that we should--this committee should at least recognize that the document remain as a draft document available to FSIS to distribute.

I would like to continue to accept comments on the document and then filter that and make some decision as to whether or not there are substantive comments that need to be dealt with on the short term, and if so, I would like to come back to the subcommittee and get consensus from them as to when and how we should make that information changed in the document available. Otherwise, I would suggest that FSIS should come back to this committee sometime after the implementation in January, such as next fall, and come forward with the charge to this committee to either expand it or improve it based on the comments, or finalize it or something to that effect.

But in any case, I think it should remain as a draft document and be made available to the very small plants at this time.

DR. POTTER: Comments from the committee?

David.

DR. ACHESON: As a member of the subcommittee, I would be reluctant to get involved in this again unless we

had a very clear mandate from the whole committee of exactly what it is that we're supposed to do.

DR. POTTER: Okay. Stephanie.

DR. DOORES: Also as a member of the subcommittee, it might be more of benefit of our subcommittee if a document were developed perhaps by FSIS or expanded by FSIS that we reacted to for our scientific input rather than our writing the document.

DR. POTTER: I think that that's the mode of operation that the committee is trying to use. So I think early in NACMCF's life, there were fewer committee members and fewer assignments, and the committee members did a lot of the writing, but there are too many assignments now.

So I believe the pattern we're trying to establish is that the agencies will do the writing, and the committee will do the reviewing.

Other comments on Dan's presentation?

DR. ENGLEJOHN: This is Dan again. In Katie's absence, she did also--I would say did object to us releasing the document early and made her opinions known about that, and certainly registered in many of her comments were incorporated into the drafts that we had, but she did make what I think is a good suggestion in that we need to have a process in place for if we have draft documents, that

we have how and when we should make them available. So I think that's a good suggestion as well.

DR. POTTER: Okay. Thanks, Dan.

Alison.

DR. O'BRIEN: For the record, I'd like to second what David said and what Stephanie said. It was very frustrating to be on that committee when we didn't really have clear guidelines about where we were supposed to be going, and I certainly can speak for Margaret who wasn't here.

DR. HULEBAK: I'll respond to that comment, Alison and David and Stephanie. The fact that the committee never really received a clear written charge was certainly an issue that I think affected the work on that document for too long, and I think we can say and Dan's comments essentially acknowledge that we've learned from that experience. As he suggested, if there is a sense that there needs to a change to the document, that that will be very clearly and plainly communicated to the committee.

On the point of draft documents, I believe that it is true that we have, essentially, procedures for handling draft documents and their availability to the public through the Federal Advisory Committee Act which specifies how documents are available to the public. Having

said that, if members of the committee want to discuss what those requirements are and have them clarified for the committee, I think that might be useful.

Mel.

DR. EKLUND: I can agree with what's been said before. I was on the subcommittee for a couple of years, and we were really frustrated for two reasons, one we didn't know where we were going with it. The second thing is that there were a number of documents already out by USDA and we didn't know how these would fit in with that. I think that was kind of the frustration of it. So it's a learning cycle. We've all learned by it.

DR. HULEBAK: And as a final note, unless anyone else has something to say, even though it was so painful and so long, what the committee has ultimately produced, the agency is finding very useful, and my sense is that the small plant operators are finding it useful too. So thank you very much. Thank you extra very much because it was so painful get there.

DR. POTTER: Bill, did you want to add some comments?

DR. SVEUM: Just a couple of minor comments.

On the topic of procedures for releasing draft reports, I don't think we need to know the details of that

as long as there is a Federal Advisory Committee procedure and perhaps we would be informed of details should such a need emerge in the future.

On the substance of this report, I was just appointed to the Meat and Poultry Subcommittee last May. So this is the very first meeting of that group that I attended. So I don't share the frustration that the other subcommittee members built up over several years working on this, but I think everybody worked very hard in May to put together a really good draft recommendation and hazard identification, and I think Dan Englejohn in particular did an extremely good job of expediting that through his editing and getting us drafts. After the meeting, we had I think three more transmissions by E-mail. So I think there's--things have really jelled and come together since last May, and a lot of good work has gone into this, and I for one would approve it as it stands today as a member of the full committee.

DR. ANDERS: I guess I would like to know from Dan a little bit more about why we need--why it cannot be finalized? Why do we need to hold it open? I mean what are we expecting to do with it?

DR. ENGLEJOHN: Well, I think that there is a recommendation--there is an understanding that the document

is fairly narrow. It doesn't deal with all the meat products and certainly can be expanded upon, and that's something, maybe, the agency can do and, I think, bring back to the committee. There would be improvements made to it, and I think we need to give it that opportunity to get the users of it to get back to us.

I mean, there are many people that reviewed it, mostly with advance science degrees, and clearly it didn't meet the level of expectation by many people, but it's really the small plant people that need to actually be able to use it, and so from that standpoint, I think it really would be beneficial to let it remain as a draft for modification, and then maybe next year at this time we can decide whether or not it should be finalized.

But I would say if it's going to be finalized, I really think that should be something that FSIS should do and bring back to the committee, and I would say there are improvements that could be made.

DR. HULEBAK: Dane.

DR. BERNARD: I would agree with that. I think we do need some time for some additional feedback. Personally, I know of three people who are preparing some feedback at the moment and that will be provided to Dan. So

I think there is some additional opportunity needed for work on the document.

E.COLI 0157:H7 BRIEFING

DR. POTTER: Okay. If there are no further comments on that, perhaps Karen would like to introduce the next topic, the E.coli 0157:H7 briefing.

DR. HULEBAK: Thanks, Morris. Now, Morris' suggestion is that Mark Powell, who I would like to introduce to you, stand on one leg for this presentation. Risk assessors are very skilled, and Mark is skilled among risk assessors, but we won't force him to do that.

Mark has recently become a full-time employee at FSIS. He came to us as a AAAS fellow and quickly demonstrated his indispensability, and we begged him to stay. He agreed. Mark has been working with a team of FSIS staff and with help from our agencies within USDA and FDA to develop a risk assessment for E.coli 0157:H7 and to take the approach that the agency has developed for considering hazards in the food supply, and that is to attempt, as much as possible, to understand the risk from farm to table.

This presents a huge challenge, obviously, for 0157. It's just as big as it is for anything else, might be greater. Mark and his team has done a terrific job, we think, and we would like for you the hear about the work

that they have done in detail, as you have been able to hear about Vibrio and Listeria at this meeting. That longer discussion of this risk assessment is going to have to wait. We may, in fact, do it in December. Mark is here today to give you an appetizer. So Mark Powell.

MR. POWELL: Well, thanks for those kind words. What I'm really going to speak to today is the hazard of taking leave. Earlier in your packets, you received copy of a presentation that I made at the OPHS meeting this past summer, and that's the very darkened copy. You should have also received a smaller handout consisting of three pages, and what I'm going to try and walk through today are the changes that we've made in our tentative characterization of the uncertainty regarding the epidemiologic-based estimates of the cases attributable to 0157 in ground beef.

The numbers have changed somewhat since the meeting as has the underlying data and the approach to some extent. So the purpose of this estimate is to provide a means of ground-truthing the results that come out of the full process risk model, the full farm-to-table model.

Now, I guess the risk skeptic in me would say, you know, why do you need to go further than just the epidemiologic-based estimates to derive an idea of what sort

of magnitude of the problem is there, but I would argue that the process risk model, the full farm-to-table model is necessary to guide your thinking about how to address the food safety problem under question throughout the farm-to-table continuum, where might there be potential critical control points, where might there be pathways of primary concern.

So again, keep in mind that the numbers that I'll be talking about today are epidemiologic-based. They're not the output on the model, but they'll be used to ground-truth the model. We need to characterize uncertainty about the epidemiologic estimates just as we characterize our uncertainty from the risk model.

What I'm going to present today is an update of the previous estimate, and this is based on work done by our colleagues at CDC and other health agencies. This is hot off the press, just released by Paul Mead and colleagues in the Journal of Emerging and Infectious Diseases. I want to acknowledge the generous help that we've received, not just in this instance from CDC and their colleagues at the state public health agencies but throughout the modelling process, and I want to congratulate Paul and his colleagues for undertaking this formidable task, and noteworthy is that in this effort is that the steps that have been taken in the

analysis allow one to really reconstruct the analysis, and it's laid out in considerable detail.

So I'll first just refer you to the handout you received previously. I believe you all have that in the dark copy. I think previously I have presented to the committee the goals of the assessment, some background, so I'll skip over much of this.

You can see the schematic of our farm-to-table process risk model. I'd like to acknowledge all the members of our team as well as the contractors that have provided valuable input and the resources, brain power that we've been able to tap through the Interagency Food Risk Assessment group that's convened by the USDA Office of Risk Assessment Cost Benefit Analysis.

The scope of the assessment is limited to ground beef at this point, and we're considering a range of public health outcomes, so I'll ask you to skip over most of this.

The first slide that I'd ask you to focus on is the epidemiologic risk factors. Kassenborg and colleagues have conducted--have some preliminary results on a case-controlled study conducted in the FoodNet catchment areas, and they've detected a population attributable risk of approximately 19 percent for consumption of pink

hamburger, and this is where we depart from the previous analysis.

So I'll just try and walk you through the changes that have been made, and I think this is really an object lesson, frankly, in how risk assessment models are dynamic beasts. They're constantly being updated as new data comes to light, and it also shows, I think, how the bottom line may not necessarily change radically when you get new information and take different approaches, and I think that that ought to give us confidence in the results, because taking different approaches gets you to the same end point.

So I'll refer you to now to the second slide, the analysis of the FoodNet surveillance that I've laid out in the second handout. So we've taken a slightly different approach. Rather than taking the simple unweighted population-based rates that FoodNet has reported, we've taken the reported weights from 1996, '97, '98 from the five primary initial FoodNet sites and weighted these rates by the cluster or state population size.

Now, I guess we might have a different interpretation as to how CDC treats this data. They have said that this has the effect or is intended to account for a hypothesized northern tier effect. It has the effect of

downweighting particular northern tier states such as Minnesota where the reported rates per 100,000 population are particularly high. I think you would have to take another approach to account for any hypothesized northern tier effect, but I think that what is being done here is weighting the sample, the catchment area by the cluster size, i.e. the state population, is appropriate, nonetheless it has the side effect of downweighting what might be considered an outlyer.

Alternatively, there might be approaches that you can take such as a robust central interval indicator for downweighting outlyers, but I think it's sufficient to say that we feel that this weighting approach is appropriate, and what this generates then is a weighted rate ranging from 1.25 to 2 cases per 100,000 population year. Now we take that reported rate, that weighted reported rate and feed into a series of steps to adjust for underreporting and also to distinguish between bloody and non-bloody diarrheal cases.

DR. HULEBAK: Mark, I'd ask you to tell us where exactly you are.

MR. POWELL: I'm on this handout. I apologize for the confusion that two sets of handouts has created,

again the hazard of taking leave. Okay. I apologize if I was unclear about which document I was referring to.

So based on the reported rates, there are recognized sources of underreporting that we want to take into account: test sensitivity, whether or not the lab performed a test on a submitted stool sample, whether the physician obtained a culture from a patient, whether or not a patient that was ill sought health care, etc., etc.

At the top of this schematic, differentiate between the bloody and non-bloody cases, we've used the FoodNet data and some data that came out of a trial in Oregon that gives us the proportion of reported cases that were bloody, 409 out of 480, and rather than treating all of these proportions that I'll be talking about as simple point estimates, these feed into beta distributions which are used to characterize our uncertainty about proportions, and this derives from statistical theory; but, essentially, if you have a beta distribution centered about 50 percent that's based on five out of ten observations, that's a broader uncertainty distribution than a beta distribution that is centered about 50 percent but is based on 50 out of 100 observations. The more information you have, the tighter the distribution is about the nominal or the point estimate,

but that gives you, I think, an intuitive feel of how we're treating these proportions.

Now each of these proportions, these beta distributions, feeds then into a negative binomial distribution which essentially yields the number of failures that you would be likely to have observed or to have missed given the number of successes you have observed. So, for example, if the number of cases, if 200 out of 250 labs surveyed for 0157 conducted the test on 0157, you could put that proportion in and estimate how many didn't test for 0157 based on the number that you have observed that did.

Okay. So the beta distribution provides the probability that inputs into a negative binomial distributions for each one of these proportions. So the proportion of bloody and non-bloody again comes from the FoodNet 1996 data as well as some data from early surveillance in Oregon, and then we utilize lab survey data that came out of the FoodNet surveillance, the additional studies that were conducted in the FoodNet catchment areas. 108 out of 230 labs surveyed test non-bloody stool samples submitted for 0158. 182 out of 230 test bloody stool samples for 0157. The test sensitivity is assumed to be 01.75. We account there for the number of false negatives.

The physician surveys, again derived from the FoodNet survey, 699 out of 1943 physicians that were surveyed obtained cultures of non-bloody--patients presenting with non-bloody symptoms, whereas 1,515 out of nearly 2,000 physicians surveyed obtained cultures when there were--when the patient presented with bloody symptoms.

Now, we depart somewhat for the proportion of patients or ill persons that seek care by utilizing the results reported by Seizlak and colleagues from a Las Vegas outbreak where the case definition was bloody diarrhea, and in this case, 32 patients out of 58 that were ill with bloody diarrhea sought care, and we used the FoodNet population survey for the proportion of cases of all non-bloody illnesses that would seek care. In that case, it was 88 out of 1,100. So we used the FoodNet survey to estimate the proportion that would seek care given that they had non-bloody symptoms.

So all of these steps then account for the underreporting and provide estimates of the total national cases of bloody diarrhea per year, and separately, both the bloody and the non-bloody cases per year. Add those, and you get the total number of cases.

For the basis of attack rates to more severe health outcomes, we consider, however, only the severe

cases, those that are bloody diarrhea and the patient seeks care, and so that outputs into the next sheet, and I'll ask you to be ambidextrous and refer to both of the handouts.

We are now on the attack rates which is slide 13. I guess I thought it was important given that there were different numbers. I wanted to explain how the new numbers came about. If you'll take it on faith, I'll gladly cut this short.

Again, I apologize for the confusion that the two separate sets of handouts.

So in order to estimate the proportion of severe cases that then progress to more severe health outcomes, such as hospitalization, HUS, or death, we've used data provided by CDC on 203 outbreaks that occurred during 1982 to '98, and you can see there the proportions of the total of number of cases that progressed to hospitalization, HUS or TTP or death, and so we've used those again, those proportions as beta distribution inputs to estimate the attack rates.

Okay. So from all sources, then-- referring back to the three-page handout--our estimated based on the-- we start with a reported, a weighted reported rate of 1.25 to 2 per 100,000 population year, make the adjustments for underreporting, and we generate a distribution of the total

number of cases. That has a confidence interval ranging from 49,000, almost 50,000, to roughly 120,000. I believe CDC's estimate is approximately 73,000 cases of 0157 from all sources, and you see that that point estimate provided by CDC lies very close to the central tendency measure that we have got here.

Breaking out the severe cases, again, those are cases in which the patient has bloody diarrhea and seeks care. We apply the proportions derived from the outbreak data to the number of severe cases generating distributions that characterize our uncertainty about the number of hospitalization cases from all sources, HUS, and death, and in each case the point estimate that is reported by CDC is very close to the median that we've reported here, but I think that the uncertainty balance gives you added information.

Now from--to derive the number of cases that we can attribute to ground beef, we need to get a handle on what proportion of illnesses are due to ground beef. So then I would refer you to one, two, three, four five slides down on the original slides, table three, identified outbreaks. Now, these are outbreaks occurring from 1994 to 1998 where the vehicle of infection has been identified. So I've taken out those outbreaks where the

likely vehicle of infection was unknown, and this data, there's a couple of different ways that you might look at it. You might look at the proportion of illnesses, outbreak illnesses associated with ground beef, or you might look at the number of outbreaks.

So in the next slide you can see--the next two slides you can see how these two different looks at the data agree or disagree. Looking just at the proportion of illnesses, there were 344 out of a total of 1,916 attributed to ground beef. This beta distribution is shown in the next slide as the very peaked distribution that is centered about 18 percent, and that seems to agree closely to the results of the FoodNet case control study by Kassenborg and colleagues that found an attributable risk of 19 percent for consuming undercooked hamburger, but our sense is that with the distribution that peaked, and basing this on outbreak data, that that distribution seems to be somewhat overconfident.

Alternatively, looking at the proportion of outbreaks associated with ground beef, a broader less confident distribution, but it seems to be somewhat biased in that is central tendency is around 32 percent which is quite a departure from the attributable risk identified through the FoodNet case control study.

So tentatively what we're doing is characterizing our uncertainty about the proportion of illnesses due to ground beef utilizing a PERT distribution which has a minimum of the 2.5 percentile to the first distribution, most likely value of the fiftieth percentile to the first distribution. That agrees very closely with the estimate from Kassenborg. And then a maximum of the 97.5 percentile of the outbreak--the distribution based on the outbreaks. That results in a PERT distribution with a minimum of about 16 percent, most likely of 18 percent and a maximum of 40 percent.

So that's how we derived the proportion or are currently characterizing the uncertainty about the proportion of illnesses due to ground beef. So then to estimate, then, the number of cases associated with ground beef, we simply apply the distribution from all sources by that fraction. Our uncertainty about that fraction is characterized as a distribution, and these generate then confidence intervals that you see in the final page of the three-page handout that I provided to you during the lunch break.

Now, consistent with CDC's findings, the results differ from the earlier estimate in that there are a higher number of cases but a slightly lower number of the

more severe outcomes such as death, and what our previous estimate wasn't capturing was a lot of the non-bloody cases that did not seek care. So, for example, if you would compare our previous estimate of the number of deaths attributable to ground beef, it was a confidence interval from--ranging from 7 to 30, and our updated estimate is a confidence interval ranging from 5 to 20 with a median of 10. So we've incorporated additional data, refining the estimate somewhat, tightened it somewhat, but it hasn't had a dramatic impact on the bottom line.

Any questions regarding the methods?

DR. HULEBAK: Art.

DR. LANG: As I'm not an expert, but I have a little bit of experience with decision modelling, and as I mentioned earlier to this group in decision analysis, at least in the models that I've used that are not the most sophisticated, it often comes down to one or two of the variables, for lack of a better word, that really drive the model.

So, for example, in a model of whether you give AZT after you stick yourself with a needle, it actually doesn't matter whether you produce a better, a more effective AZT, you know, antiviral drug in terms of the

outcome of the model. What drives that decision, if you just go by the numbers, is the probability of getting HIV.

So I guess the question for me would be now that you've actually used this model, are there particular variables that the model is particularly sensitive to? Because it would appear that it was wasn't terribly sensitive--it didn't seem to matter that much--the improvement in the estimate that you got from Paul Mead didn't seem to matter that much in the bottom line if I understand.

MR. POWELL: All right. Well, again, I want to underscore that this is our characterization of our uncertainty about the empirical data and not the model. This is the empirical information that we'll use to ground-truth the results of the model. You could, however, conduct some sensitivity analysis just on this analysis of the epidemiologic data that may provide you some insight as to what you might want to reduce uncertainty in your surveillance-based estimates.

So, for example, it might--you know, and I haven't had the opportunity to do it, but it might be the case that the--you know, the proportion of bloody versus non-bloody cases is kind of a driving uncertainty factor, in which case you might look there to better refine your

estimate there, and as I said, the beauty of these uncertainty analysis methods is that the more data you gather, your bounds of uncertainty tighten, and so you could perform that sort of analysis on this, but it's not the model.

This is the empiric observation that we're trying to adjust for the shortcomings of empiricism.

DR. POTTER: Other comments? Okay. Stay tuned for a more full disclosure of this or full description of this in December.

DR. HULEBAK: And we'll try to schedule it for the morning. What Mark and the team have done is impressive and very complete, and it's tough to listen--I mean it's tough to convey that kind of information to a group after the end of a long day. So thank you all for your attention. Dane.

DR. BERNARD: I just woke up.

[Laughter.]

DR. BERNARD: So you can thank everybody else for their attention, but I just woke up.

Farm-to-table risk assessment, and I know this isn't the total body of work here, but I don't notice things like contamination rates coming into the slaughter house, contamination rates on carcasses having been subjected to

various treatments. Is that in the process? It seems like what you've done so far is take existing data and tried to predict basic outcome on the finished product or near finished product.

MR. POWELL: Again, this is not the results of the model. This is trying to characterize our uncertainty about the data that we'll use to validate the model. Okay. We're using an independent set of data, the epidemiologic data derived from FoodNet and outbreak data. Okay. Setting that aside, as good modelers should, and going ahead and performing our, you know, process risk model with the sort of data that you're talking about, and then hopefully at the end they will agree, but this is the information that we're using to validate the output of the farm-to-table model.

DR. HULEBAK: So the model that--the actual model includes factors of the sort that you've mentioned.

MR. POWELL: We'll be predicting the same sorts of things that I'm talking about here. Okay. And we'll have more confidence in the output of the model if it agrees with these epidemiological-based estimate, but we still need to take into consideration the uncertainty into the epidemiologic-based estimate, and that perhaps is why it seems like a modelling exercise, because we're using those

sorts of methods to characterize or our uncertainty about the observational data. You're puzzled.

DR. BERNARD: We'll talk later.

MR. POWELL: Okay.

DR. HULEBAK: Any other questions?

FUTURE ACTIVITIES

DR. POTTER: Okay. We're now ready for future activities. I suspect that Karen will have some things for the committee. I'd like to remind the committee members that we will be meeting December 8, 9, and 10 here in Washington for our next session. So if you could sort of block those days on your calendar, we'd appreciate it.

If Kay had been able to stay, she wanted to discuss the choice of colors for the official NACMCF blazers and choice of fanfare for the grand entrance and seating of the executive committee, but that will have to wait for the next meeting.

DR. MORALES: Put that as a morning agenda item.

DR. HULEBAK: I actually didn't have anything to lay before the committee at this time as immediate future activities. I think it's got quite a menu before it in the next few months.

I will add one update about an activity going on outside this committee's work that will probably in the future be interesting to you, and that is the antimicrobial resistance action plan development that I mentioned at our last meeting. That work has been continuing. There's a draft plan developed. It's anticipated that plan will be available for public comment the first part of the year. I still think that out of that action plan may come issues that this committee is going to be interested in discussing, but that really is future. That's more than a year off for this committee.

Having said all of that, we finished I'd say almost an hour and a half ahead, more than an hour and a half ahead of time. I'm tempted to adjourn.

Are there any issues, any other issues, thoughts or parting words that any of the committee would like to make?

DR. POTTER: On behalf of FDA, I'd like to thank you all very much for your hard work on some issues that are very important for FDA in particular, but administration in general. So thank you very much.

DR. HULEBAK: Thank you, and--oh. Mel.

cc

DR. EKLUND: I just wanted to thank Morris for all the work he's done on the committee, and we wish him well at his new job.

[Applause.]

DR. HULEBAK: You'll have to wear the NACMCF blazer.

Thanks a lot. See you in December.

[Whereupon, at 3:53 p.m., the meeting was adjourned.]

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