codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 2b) **CX/FFP 03/2-Add.1**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS

Twenty-sixth Session
Ålesund, Norway, 13 - 17 October 2003

MATTERS REFERRED TO THE COMMITTEE

MATTERS ARISING FROM FAO AND WHO: MICROBIOLOGICAL RISK ASSESSMENT OF *VIBRIO* SPP

1. BACKGROUND

1. At the twenty fifth session of the Codex Committee on Fish and Fish products, FAO and WHO informed the committee of their activities on risk assessment of *Vibrio* spp. in seafood. This work was being carried in response to a request from the Codex Committee on Food Hygiene. In discussing this issue the CCFFP identified a number of questions in relation to management strategies for food-borne illness due to *V. parahaemolyticus* and *V. vulnificus* and which could contribute to the "proposed draft standard for live, quick frozen and canned bivalve molluscs". These were addressed to FAO and WHO to be considered as part of the risk assessment work. These questions were considered by an expert consultation on risk assessment of *Vibrio* spp. in seafood and *Campylobacter* spp. in broiler chickens that was held in the FAO Regional Office for Asia and the Pacific in Bangkok in August 2002. The questions were further considered by the expert drafting group on risk assessment of *Vibrio* spp. in seafood. A response to these four questions is provided below. This is based on the expert opinion of the participants of the expert consultation that was held in Bangkok and the work being done as part of the FAOWHO risk assessment on *Vibrio* spp. in seafoods.

2. RESPONSE TO THE QUESTIONS POSED BY THE CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS

2. **Question 1:** Are the following pre-harvest control measures (testing/monitoring the following parameters and consequential closure of the harvesting area) effective in the control of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in bivalve molluscs:

- Testing of bivalve mollusc meat for *Vibrio parahaemolyticus* and *Vibrio vulnificus*
- Temperature monitoring of the growing water
- Water testing for *Vibrio parahaemolyticus* and *Vibrio vulnificus*
- Salinity monitoring

3. **Response:** The concentrations of *V. parahaemolyticus* and *V. vulnificus* in shellfish may be measured directly or predicted by monitoring temperature and salinity. There will not necessarily be a direct relationship between these surrogate variables and the measured concentrations of pathogenic vibrios for a particular area as there is uncertainty and variability in the current models. The predictive abilities of the models would be improved by incorporating local data and considering additional factors such as hydrodynamic effects and sunlight. The effectiveness of these measures in controlling illness would depend on the instigation of an appropriate mitigation (or multiple mitigations) and this is not confined to closure of a harvesting area.

4. The current models do not include modules relating to the concentration of these two pathogens in seawater and thus the utility of measuring this cannot be estimated. If the appropriate data were gathered then the models could be extended accordingly.

5. **Question 2** Are the following post-harvest treatment technologies, alone or in combination, effective in the reduction or elimination of *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs:

- hydrostatic pressure
- rapid cooling
- irradiation
- mild heat treatment (pasteurization)
- freezing and thawing
- depuration

6. **Response**: These may all have the effect of reducing the numbers of pathogenic vibrios but the effectiveness will vary according to the conditions of use, and there may be a need to balance between obtaining the maximum possible reduction in bacterial content and retaining consumer-acceptance of either the product or the process. A qualitative assessment of the technologies which may reduce *Vibrio* contamination in oysters was undertaken (Table 1). A literature review was also undertaken to try and determine the effectiveness of these mitigations I more quantitative terms. Some of the mitigations are also being considered and evaluated in the FAO/WHO risk assessment on Vibrio spp. in seafood that will be available in 2004.

Table 1: The comparative effectiveness of a number of mitigation strategies in reducing *Vibrio* spp

Mitigation	***Comparative effectiveness in reducing Vibrio spp.***
Hydrostatic pressure	+++
Rapid cooling	+/++
Irradiation	+++
Pasteurization	+++
Freezing and thawing	++
Depuration	+/-
Relay at high salinity	++
Commercial heat-treatment	+++

\- no effect
\+ some reduction
++ moderate reduction
+++ significant reduction

7. **Regulatory settings surrounding mitigations:** In recent years, at least two countries, Japan and the United States of America have regulated to reduce the impact of vibrios in shellfish. In Japan, regulations effectively prevent harvest of oysters during warm months by stipulating a limit of 100 MPN/g *V. parahaemolyticus* in seafood intended for raw consumption. In the USA, the National Shellfish Sanitation Program (NSSP) time/temperature matrix for *V. vulnificus* requires oyster harvesters from any state which has previously had two or more confirmed cases of *V. vulnificus* to refrigerate oysters within 10 hours after harvest during summer months, depending on water temperature. The net effect of this regulation is a 10-fold reduction in *V. parahaemolyticus* levels, compared to not refrigerating until 20 hours after harvest.

8. For *V. parahaemolyticus*, the Interstate Shellfish Sanitation Conference (ISSC) in the USA implemented an Interim Control Plan, based on monitoring when and where historical episodes occur; detection of pathogenic *V. parahaemolyticus* (tdh+) in oysters results in cessation of harvest until monitoring indicates the pathogen is no longer detected.

9. As well as regulations to prevent growth of vibrios or harvesting when levels are high, there are numerous interventions which reduce levels of vibrios in oysters. Almost all the mitigations have been developed in the USA against a background of increased concern regarding *V. vulnificus*, in particular. The

shellfish industry, through the ISSC, has sought approaches to minimizing illnesses caused by vibrios. These include education for at-risk individuals, limiting shellfish harvest during certain periods of the year, minimizing time between harvest and refrigeration of shellfish and post-harvest treatment (PHT) of shellfish.

10. The harvesting practices and post-harvest interventions considered here include:
Rapid cooling
High hydrostatic pressure processing
Irradiation
Hot water/cold shock pasteurization
Freezing
Relaying at high salinity
Depuration

11. The standard for effective PHT set by the ISSC is a 5-log reduction of vibrios with an endpoint of non-detectable (<3 MPN/g for *V. vulnificus* and <10 CFU/g for *V. parahaemolyticus*). In the USA, industries that treat shellfish to these levels are allowed to use a safety-based labelling claim on their product.

12. **Rapid cooling**: Vibrios grow within the meat and liquor of some oysters. In the Eastern oyster, *C. virginica*, *V. vulnificus* and *V. parahaemolyticus* densities increase 10-100-fold in 10 h at typical ambient temperatures on the United States Gulf Coast between April and October. For this reason, rapid cooling has been identified as a control point in preventing multiplication and as a regulatory guideline (ISSC Model Ordinance) so that, when the harvest water temperature exceeds 28°C, oysters harvested from waters of states with 2 or more confirmed *V. vulnificus* illnesses must be placed under refrigeration (10°C) within 10 h of harvest (DHSS, 1999).

13. Not all oysters support the growth of vibrios after harvest. For example, the Sydney Rock Oyster (*Crassostrea commercialis*) has a commercial shelf-life of some weeks at ambient temperatures (15-20°C) implying that bacterial numbers do not increase significantly.

14. Refrigeration temperatures not only prevent growth, but have a vibriocidal effect, with the effect of chilling oysters over a period of 14 days causing a 0.8 log reduction (Gooch *et al.* 2002).

15. The effect of rapid cooling is also considered in the FAO/WHO *V. vulnificus* risk assessment which will be published in early 2004. Currently a summary of the work is available in the report of the FAO/WHO expert consultation on Risk Assessment of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood that was held in Bangkok in August 2002. This is available on the FAO and WHO webpages (ftp://ftp.fao.org/es/esn/food/cv_02e.pdf and http://www.who.int/fsf/Documents/Bangkok_Campy_02_En.pdf)

16. **Hydrostatic pressure:** High Pressure Processing (HPP) also known as Ultra High Pressure (UHP) is finding use as a means of reducing vibrios in oysters to non-detectable levels (Table 2). Equipment is now available for processing relatively large volumes (150L) of product. Application of 200-300mPa for up to 300s to oysters has been shown to effect a 3.5-6 log reduction in vibrios without any reduction in sensory quality (Berlin *et al.*, 1999; Calik *et al.*, 2002: Cook, in press).

Table 2: Effect of processing time and pressure on vibrios in naturally-contaminated oysters

	Pressure/time (mPa/s)	Log reduction	Reference
V. vulnificus	241/120	>4.8	Cook (in press)
	200/600	6	Berlin *et al.* (1999)
V. parahaemolyticus	275/180	3.5	Cook (in press)
	300/120	4	
	345/30	6	Calik *et al.* (2002)

17. High pressure treatment has become a commercial decontamination process for oysters in the USA and Australia. For oysters from the Gulf of Mexico pressure/time combinations are used to effect a 5-log reduction of *V. vulnificus* while, in Australia, 265mPa/45s for shucking and 265mPa/180s for "shelf-life extension" are used. There have been no studies on reduction in contamination levels of vibrios in oysters in Australia but it is considered that the pressure/time combination effects a 5-log reduction in vibrios.

18. **Irradiation:** Doses of 3kGy have been shown to eliminate *V. cholerae* from frozen shrimp (Rashid *et al.* 1992; Ito *et al.* 1993). More recently, Jakabi *et al.* (2003) have confirmed that 3kGy delivers a 5 to 6-log reduction in *Salmonella* Enteritidis and *V. parahaemolyticus* in unshucked oysters. The treatment apparently led to no loss in sensory quality and the oysters remained alive.

19. **Hot water/cold shock pasteurization:** Low temperature pasteurisation has proven effective in eliminating vibrios from oyster meat (Cook and Ruple, 1992) and from shell-stock oysters where a 5-log reduction in *V. vulnificus* and *V. parahaemolyticus* was also obtained in live, shell-stock oysters from the Gulf of Mexico using 50°C for 10-15 minutes (Andrews *et al.*, 2000). The same authors also demonstrated a die-off of vibrios in ice storage, though the time was probably longer than that usual in the marketing chain. There was no difference in sensory quality between pasteurised and raw oysters.

20. More recently, Andrews *et al.* (2003) studied the effect of the technique on *V. parahaemolyticus* O3:K6, a pathogenic strain with enhanced heat resistance. The researchers found that 6 minutes heating at 50-52°C reduced a 4 log contamination level to undetectable levels (<3 MPN/g). When the pathogen was present at levels of 5-6 logs, a heating time of 22 minutes was required to reach non-detectable levels. The authors note that, while the O3:K6 strain has not yet been isolated from waters in the USA, it nonetheless was implicated in the 1998 outbreak involving oysters from Galveston Bay in the USA.

21. A technique involving dipping oysters in tanks with water at 67°C for around 5 minutes followed by spraying with cold water for around one minute to assist in shucking was described by Hesselman *et al.* (1999). When combined with market chain procedures such as chilling, packing and cold storage, *V. vulnificus* was reduced by 2-4 logs, depending on the original contamination level, but was not sufficient to reduce levels to non-detectable (<0.3 MPN/g).

22. The technique has also proved effective in other bivalves. Cockles (*Anadara granosa*) heated in water at 99°C achieved an internal temperature of 42-58°C after 10s and 56-69°C after 30s, the latter effecting a 5-log reduction in *V. cholerae* inoculated into the cockles (Liewe *et al.* 1998). Note that in Thailand, *A. granosa* is also known as Bloody Clam and details of a risk assessment of *V. parahaemolyticus* in Bloody Clams are included in the FAO/WHO risk assessment on Vibrio spp. in seafood..

23. **Freezing and thawing:** It is well known that vibrios, in general, do not survive well at temperatures below their growth range At refrigeration temperatures they undergo cold shock while freezer temperatures provide a second element of inactivation. Muntada-Garriga *et al.* (1995) followed inactivation of *V. parahaemolyticus* in oyster meat at various commercial cold storage temperatures and found die-off over time, which was more rapid at freezer, compared with refrigerator, temperatures. The study has most relevance for frozen oyster meat stored for some months and is unlikely to reduce the organism to undetectable levels in shellstock over normal marketing times at refrigeration temperatures (<2 weeks).

24. Freezing and frozen storage also reduced numbers of *V. vulnificus* in oyster meat by 3-4 logs over 70 days, with the organism dying more rapidly in vacuum, compared with conventional packaging (Parker *et al.*, 1994). Cook and Ruple (1992) studied survival of *V. parahaemolyticus* in oysters at -20°C; there was a 2-log reduction over 30 days, with both pathogenic and non-pathogenic strains responding similarly to frozen storage. The effect of freezing and thawing is also considered in the FAO/WHO risk assessment on *V. vulnificus* in oysters.

25. **Relaying at high salinity:** In Australia, Son and Fleet (1980) demonstrated the effectiveness of reducing pathogenic bacteria from oysters (*C. commercialis*) relayed from estuarine to high-salinity waters. In the USA, Kaspar and Tamplin (1993) demonstrated that when salinity fell below 25ppt, *V. vulnificus* numbers increased in seawater whereas, at high salinities (30-38ppt), numbers decreased by 1-2 logs. The effect of relaying oysters in high salinity has also been demonstrated by Motes and DePaola (1996), these

authors showing that relaying *C. virginica* from low to higher salinity (30-34ppt) for 1-2 weeks effected a 3-4 log reduction. Relaying as a mitigation step is also considered in the FAO/WHO risk assessments on *V. parahaemolyticus and V. vulnificus* in oysters.

26. **Depuration:** Depuration is a process that enables shellfish to eliminate microorganisms that are ingested as food, through a process of "flushing" the feeding and digestive system of the mollusc with uncontaminated water. Vibrios are part of the natural microbiota of shellfish and are often found in the viscera, haemocytes and mantle tissues of oysters (Olafsen *et al*., 1993). Consequently, depuration is relatively ineffective in eliminating *Vibrio* spp. from bivalve molluscs (Tamplin and Capers, 1992).

27. Depuration has been shown to be ineffective in reducing *V. parahaemolyticus* and *V. vulnificus* from naturally-contaminated Sydney rock oysters (Eyles and Davey, 1984). Desmarchelier (1997) cites reports showing that depuration is ineffective against *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Jackson and Ogburn (1996) provide a detailed review of the subject, supporting the above general conclusions. The same authors also report that several studies indicate increases in *Vibrio* numbers in shellfish during depuration. Depuration is considered in the FAO/WHO risk assessments on *V. parahaemolyticus and V. vulnificus* in oysters.

28. **Summary of effectiveness of inactivation technologies:** Table 3 presents a summary of the level of inactivation of vibrios in oysters which may be expected to result from using the interventions described above.

Table 3: Inactivation of vibrios in oysters by intervention technologies

Inactivation	***Log reduction***	***Comment***
High pressure processing	4-5	
Irradiation	>5	
Hot water/cold shock pasteurization	5	4-log reduction obtained for O3:K6 strain
Freezing	3-4	
Relaying at high salinity	3-4	
Depuration	None/uncertain	

29. **Conclusions:** The above review indicates that several commercial practices and interventions exist which mitigate the risk of vibrio infections from raw oyster consumption. Firstly, the practice of chilling oysters soon after harvest prevents a 1-2 log increase in those species which support bacterial growth e.g. *C. virginica*. Then, interventions such as high pressure processing or low temperature pasteurisation effect a 5-log reduction. Relaying and freezing also effect significant inactivation of contaminating vibrios. Finally, refrigerated storage in the marketing-food service-consumption chain may be expected to provide a further reduction approaching 1 log.

30. The net effect is that practices and interventions exist which, in combination, can effectively eliminate the risk of *Vibrio* infection in vulnerable consumers from oysters harvested even in warm months from waters where pathogenic vibrios are naturally-occurring. It follows that such practices and interventions should be incorporated into food safety plans by harvesters and processors. For steps which operate as Critical Control Points (CCPs) it is necessary to first validate its effectiveness, and then to verify the delivery of the CCP during the process.

31. **Question 3** For *Vibrio parahaemolyticus* - Are food-borne illnesses caused by the heat resistant toxin produced by the pathogen or by the pathogen itself?

32. **Response:** The illness is caused by the toxin but only if this is produced in the intestine following colonization by a strain producing TDH, TRH or both toxins.

33. **Question 4**: What is the availability of methods of analysis for the *Vibrio parahaemolyticus* toxin gene (*tdh*)?

34. **Response:** Both *tdh* and *trh* genes can be detected using PCR with relevant primers and by membrane filtration-hybridization methods with non-isotopic oligonucleotide or PCR-generated probes. For quantification, PCR methods can be applied in an MPN format whereas membrane filter-hybridization can be used for direct colony enumeration. PCR and colony hybridization procedures are also available for the thermolabile haemolysin gene (*tlh*) for determining *V. parahaemolyticus* species. As with conventional methods, there is scope for standardization and/or the determination of the relative performance of current methods.

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