Memorandum

Date: January 2, 2003

From: HFS-255: Robert I. Merker, Ph.D.

Subject: FAP 9M4682: Microbiological Review

To: HFS-255: Lane A. Highbarger, Ph.D., Consumer Safety Officer

Background

The National Fisheries Institute and the Louisiana Department of Agriculture and Forestry submitted a food additive petition entitled "Ionizing Radiation for The Pasteurization of Fresh or Frozen Molluscan Shellfish," which was received by FDA on June 29, 1999, and designated FAP No. 9M4682. The petitioners seek to amend 21 CFR Part 179.26: "Ionizing Radiation for the Treatment of Food" to include the following:

> Use 10. To control *Vibrio* in fresh shellfish in a single treatment in the final packaging.
> Limitations:
> Shellfish must have been properly tagged and maintained at the required temperatures (i.e., NSSP) from time of harvesting until time of irradiation and return to vendor.
> Shellfish must have been properly washed and cartoned prior to irradiation.
> Labeling:
> To the existing label shall be added in a prominent and conspicuous area the international symbol for irradiation [as defined in Part 179.26 (c)(l)] along with either the statement "Treated with radiation" or "Treated by irradiation." In addition other statements that give the reason for or benefit of irradiation may be allowed (such as "Treated by irradiation to destroy *Vibrio* and other bacteria such as *Listeria* and *Salmonella* that may cause illness").

While the submission originally set a limit of 7.5 kiloGray (kGy) as the maximum irradiation dose, and 0.5 kGy as the minimum, the petitioners, in an amendment dated April 21, 2001, requested that the regulation limit the maximum dose to 5.5 kiloGray (kGy) and removed the minimum level. This memorandum will focus on issues relevant to the use of irradiation as a physical process for the elimination of *Vibrio* spp. and reduction of existing *Salmonella* and *Listeria* species in fresh or frozen molluscan shellfish.

The Problem:

Vibrio species predominate in estuarine environments; consequently, they are naturally present in molluscan shellfish, sometimes at high levels. Studies have clearly detected vibrios in most finfish and shellfish (Buck, 1998, cited in Oliver and Kaper, 2001). Most cases of reported diseases attributed to vibrios are associated with recent consumption of seafood, particularly raw oysters. In general, vibrio populations increase both in waters and in shellfish from April to October. While *Vibrio* spp. from shellfish infect relatively few individuals, diseases vibrios

cause may be severe and become lethal. Of twelve *Vibrio* species known to cause human infections, eight have been associated with consumption of food. *V. parahaemolyticus* and *V. vulnificus* are most commonly isolated from raw and cooked oysters. *Vibrio vulnifcus* causes severe foodborne infections and is associated with 95% of all seafood-borne deaths in the U.S. In contrast to diseases associated with most vibrios, infections from *V. vulnificus* rarely result in gastroenteritis (summarized in Oliver and Kaper, 2001). Infective doses of vibrios depend upon the health of the infected individual and the particular bacterial strain. In most reported cases of disease from *V. vulnificus*, the affected individuals have an underlying chronic disease. The incubation period for diseases caused by vibrios after ingestion of contaminated raw or partially cooked shellfish ranges from a few hours to several days (Oliver and Kaper, 2001)

Disease outbreaks attributed to *V. parahaemolyticus* in raw oysters in 1997 and 1998 resulted in increased attention to this pathogen from FDA and other public health agencies. FDA recently conducted a risk assessment for *V. parahaemolyticus* in raw oysters. A draft report of this risk assessment was announced in the Federal Register (66 FR 5517) and made available on January 18, 2001 (see http://www.cfsan.fda.gov/~dms/fs-toc.html). The draft risk assessment evaluated factors that affect the prevalence of *V. parahaemolyticus* in oysters before and after harvesting. It also assessed several preventive and intervention measures aimed at reducing the incidence of *V. parahaemolyticus* in oysters, including the Interstate Shellfish Sanitation Conference (ISSC) guidance of limiting viable *V. parahaemolyticus* to 10,000 or fewer cells per gram of seafood.

A recent General Accounting Office audit for the FDA Seafood HACCP program (available from the GAO Internet site at http://www.gao.gov/new.items/d01204.pdf) concluded that while progress has been made in FDA's Seafood HACCP Program, the program does not yet prevent the distribution of unsafe seafood. FDA responded to this audit with intensified inspections and more frequent testing for pathogens. Problems with storage temperatures in warehouses were brought forward as a significant concern in the audit.

Safety Aspects relating to Microbiology in FAP 9M4682

Reduction of *Vibrio* spp.

In general, the petition relies upon published or other publicly available information or material from previous food additive petitions to address safety issues. In the amendment, the petitioner proposes a maximum dose of 5.5 kGy in molluscan shellfish for elimination of *Vibrio* and control of *Salmonella* and *Listeria.*

The petitioner has documented that *Vibrio* spp. in uncooked molluscan shellfish provide a significant public health risk. Vibrios are highly sensitive to irradiation; they usually are eliminated by doses as low as 0.5 kGy. Published D_{10} values for *Vibrio parahaemolyticus* and other vibrios range from 0.022-0.357 (Tarkowski, 1971 as cited by Farkas, 1998). Dixon, (Master's thesis, 1992) expresses concerns that irradiation process may not be effective as a means to extend shelf life for the following reasons: (1) dosimetry measurements in the oyster indicate that only about fifty percent of the irradiation dose penetrates the shell; (2) viable but

nonculturable cells (so-called VBNC state) of *V. vulnificus* may survive, but not be enumerated; and (3) oysters were sensitive to two kGy irradiation and died. However, while Dixon found that the D_{10} values for the VBNC increased significantly from those of exponential phase cells, irradiation would remain effective for a transient short term reduction of the numbers of vibrios in molluscan shellfish. Importantly, however, temperatures must be properly controlled even when infected oysters are irradiated.

Control of *Salmonella* and *Listeria*

Control of contaminating *Salmonella* or *Listeria* generally requires higher doses than for vibrios, likely about 0.5-1.0 kGy and 0.4-0.64 kGy respectively. Several publications found in the petition state that these genera can be reliably eliminated by doses well under 10 kGy. Diehl (1990) states that a dose of 5 kGy will reduce a population of *Salmonella* serotypes, *Staphylococcus aureus,* Shigella, ..., and *Vibrio* by at least six log cycles. Tauxe, in a recent review (2001), reports 5-log reductions for *Listeria* and *Salmonella* at 2.25 kGy and 2.80 kGy. D_{10} values for irradiation cited in published literature for several *Salmonella* serotypes in various fresh foods ranged from 0.22 to 0.921 kGy. Similarly, reported D_{10} values for *Listeria monocytogenes* in fresh meats and vegetables ranged from 0.27-0.77. Therefore, the dose limit in the proposed regulation could significantly reduce the populations of these organisms as well, even considering concerns about penetration.

Concerns about *C. botulinum* and Botulinum toxin in molluscan shellfish

The petitioners acknowledge that *Clostridium botulinum* type E could be a concern for seafoods. Among other references that they provide, Diehl (1990) provides an in-depth discussion of the likelihood for outgrowth and toxin production by *C. botulinum* type E. He cites studies in his laboratory on the effect of storage temperature and irradiation on toxin production by *C. botulinum* type E. Although these studies dealt with fish rather than shellfish, and dosage allowances should be adjusted for certain aspects of these products (e.g., shells); in general, the same principles should apply. In these studies, no toxin was detected after incubation with fish of up to 10^5 organisms at 0°C for eight weeks, well beyond the shelf life of these products. At 5°C, no toxin was produced for up to six weeks of storage in untreated cells, and for up to seven weeks when irradiated at 2 kGy. As seven weeks is far beyond the expected shelf life of fresh seafood, toxin would not be produced under these conditions. However, at 10°C, detectable toxin could be detected at one week in fish inoculated with 10^5 organisms subjected to 1 kGy irradiation, and at two weeks when subjected to 2 kGy irradiation. Consequently, although the conditions in this experiment were exaggerated (10^5 organisms is higher than would be expected to survive, and 10°C for over a week constitutes significant temperature abuse), it is critically important that low temperature be maintained for seafoods irradiated under these conditions. Given that HACCP plans currently in effect for such seafoods require storage under proper conditions and that the proposed regulation emphases proper handling, irradiation would serve as an effective method for the primary intended use of eliminating populations of vibrios in molluscan shellfish without adding a significant hazard from promoting the growth of and toxin production by *C. botulinum* Type E.

Conclusions

This petition includes data and information that support the effectiveness of the proposed irradiation at a maximum level of 5.5 kGy, as well as data and information that indicate that additional controls should be implemented even when irradiation is used to reduce numbers of vibrios. In general, while irradiation may be used to reduce the levels of vibrios and other bacteria, information in the petition indicates that such treatment would not significantly prolong shelf life. In its effects on the shellfish, irradiation may actually shorten the shelf life of oysters or clams by killing them. The petition also includes information that supports the premise that when appropriate HACCP procedures and storage controls are in place, there should be no significant risk of toxin production from germinated spores of *C. botulinum* Type E.

Based on the data submitted in the petition and information in our files, we conclude that the proposed use of irradiation will not create additional microbiological hazards, and that the treated molluscan shellfish will be at least as safe as untreated shellfish currently on the market. Therefore, from a microbiological standpoint, we would support approval of the petition.

Robert I. Merker, Ph.D.

References

1. Farkas, J. 1998. Irradiation as a method for decontaminating food. A review. Int J Food Microbiol 44:189-204

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4. Oliver, J.D. and Kaper, J.B., 2001. Vibrio Species. *In* M.P. Doyle, L. Beuchat and T.J. Montville (ed.) *Food Microbiology, Fundamentals and Frontiers, 2nd Ed.,* ASM Press, Herndon, VA.

5. Dixon, W.D. 1992. The effects of gamma radiation (^{60}Co) upon shellstock oysters in terms of shelf life and bacteria reduction, including *Vibrio vulnificus* levels. M.S. thesis. University of Florida, Gainesville.

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