Effect of Gamma Irradiation on Shelf Life and Bacterial and Viral Loads in Hard-Shelled Clams (*Mercenaria mercenaria*)

PATRICK HAREWOOD,[1] SCOTT RIPPEY,[1]* AND MARY MONTESALVO[2]

Northeast Seafood Laboratory, U.S. Food and Drug Administration, Davisville, North Kingstown, Rhode Island 02852,[1] and Radiation Laboratory, University of Lowell, Lowell, Massachusetts 01854[2]

The feasibility of using ^{60}Co gamma irradiation to inactivate total coliforms, fecal coliforms, *Escherichia coli*, *Clostridium perfringens*, and F-coliphage in hard-shelled clams, *Mercenaria mercenaria*, was investigated. The results of three trials indicated average D_{10} values of 1.32 kGy for total coliforms, 1.39 kGy for fecal coliforms, 1.54 kGy for *E. coli*, 2.71 kGy for *C. perfringens*, and 13.50 kGy for F-coliphage. Irradiation doses of >0.5 kGy were significantly lethal to the shellfish.

For more than 40 years, the effects of irradiation on the physical and chemical properties of food, as well as the safety, nutritional quality, and consumer acceptability of irradiated foods (7, 16), have been investigated. Food irradiation has been studied for its value as a preservation method which reduces the number of spoilage organisms and as a disinfection method which inactivates pathogenic microorganisms in shellfish (5, 9, 12, 15).

Pathogenic agents of significant public health concern to the National Shellfish Sanitation Program include naturally occurring marine *Vibrio* species and Norwalk and other small, round-structured viruses that are introduced into saline environments by wastewater. The viral agents have been documented or implicated in many cases of gastroenteritis in the past 2 decades among consumers of raw molluscan shellfish (13). Strategies that have been investigated to address this problem include the examination of alternative indicator organisms for more reliable classification of shellfish-growing waters and the further evaluation of established procedures, including depuration or relocation (relay) of shellfish (currently used for eliminating bacterial contaminants), to reduce viral levels in shellstock after their initial harvest. There is an ongoing interest in examining all economically feasible purification procedures, including irradiation, to produce a safer product.

Studies (10–12) using inoculated, homogenized shellfish meats from soft-shelled clams (*Mya arenaria*) irradiated at exposures of ≤1 kGy (1 kGy = 100,000 absorbed radiation units) demonstrated a 100- to 1,000-fold reduction in the numbers of *Escherichia coli* (D_{10} = 0.37 kGy), *Salmonella typhimurium* (D_{10} = 0.51 kGy), *Staphylococcus aureus* (D_{10} = 0.42 kGy), and *Streptococcus faecalis* (D_{10} = 0.97 kGy) organisms. Doses of >1 kGy significantly decreased shellfish survival. Radiation studies have also been conducted with mantle fluid from *Crassostrea virginica* inoculated with certain *Vibrio* species (10). D_{10} values of 0.04 kGy were calculated for *Vibrio cholerae* O1 (El Tor), 0.06 kGy for *V. cholerae* (non-O1), and 0.07 kGy for *Vibrio vulnificus*. In a later study (8) using live

oysters, doses of ≤1 kGy were lethal to *V. cholerae*, *V. vulnificus*, and *Vibrio parahaemolyticus*; however, the D_{10} values were not specified. These oysters survived radiation exposures of <2.5 kGy, although the duration of survival was not stated.

Studies using gamma irradiation have been conducted on the inactivation of viral pathogens in shellfish (10, 11). D_{10} values of 2.02 kGy for hepatitis A virus in live oysters and 3.30 kGy for poliovirus I in live, hard-shelled clams were determined.

The present study reports the effect of gamma irradiation on the shelf life and the bacterial and viral loads in hard-shelled clams, *Mercenaria mercenaria*. In vivo inactivation rates were determined for traditional and alternative microbial indicators.

Approximately 250 hard-shelled clams (*M. mercenaria*) were harvested from Allen Harbor, Davisville, R.I., for each trial. The shellfish used were in the "little neck" category, i.e., of a size that would be consumed raw on the half shell. They ranged in diameter from 2.7 to 3.9 cm and in shell thickness from 2.3 to 4.5 mm (average, 3.1 mm). Before contamination, the shellfish were refrigerated for 48 h at 4°C to enhance subsequent pumping activity. They were then placed in tanks containing 150 liters of seawater (salinity, 30‰), supplemented with raw sewage to attain a targeted microbial indicator density of approximately 2.0×10^4 fecal coliforms per 100 ml. During this 48-h contamination process, raw sewage was added to the tank at a rate of 3 ml/min with no exchange of seawater. Microbial indicator densities were determined according to the procedure of Dufour et al. (6). The temperature of the tank was maintained at 15.5°C. After 48 h, the shellstock were placed in plastic bags on ice before the irradiation trials.

After contamination, the shellstock were transported on ice (within 2 h of collection) to the radiation laboratory at the University of Massachusetts at Lowell, where they were exposed to gamma irradiation from a cobalt-60 source for various time intervals or left untreated.

Approximately 25 shellfish were placed in a single layer and sealed in a polyethylene bag. Four bags were then placed in a rectangular arrangement between two sheets of cardboard

---

* Corresponding author. Mailing address: FDA Northeast Seafood Laboratory, Davisville, North Kingstown, RI 02852.
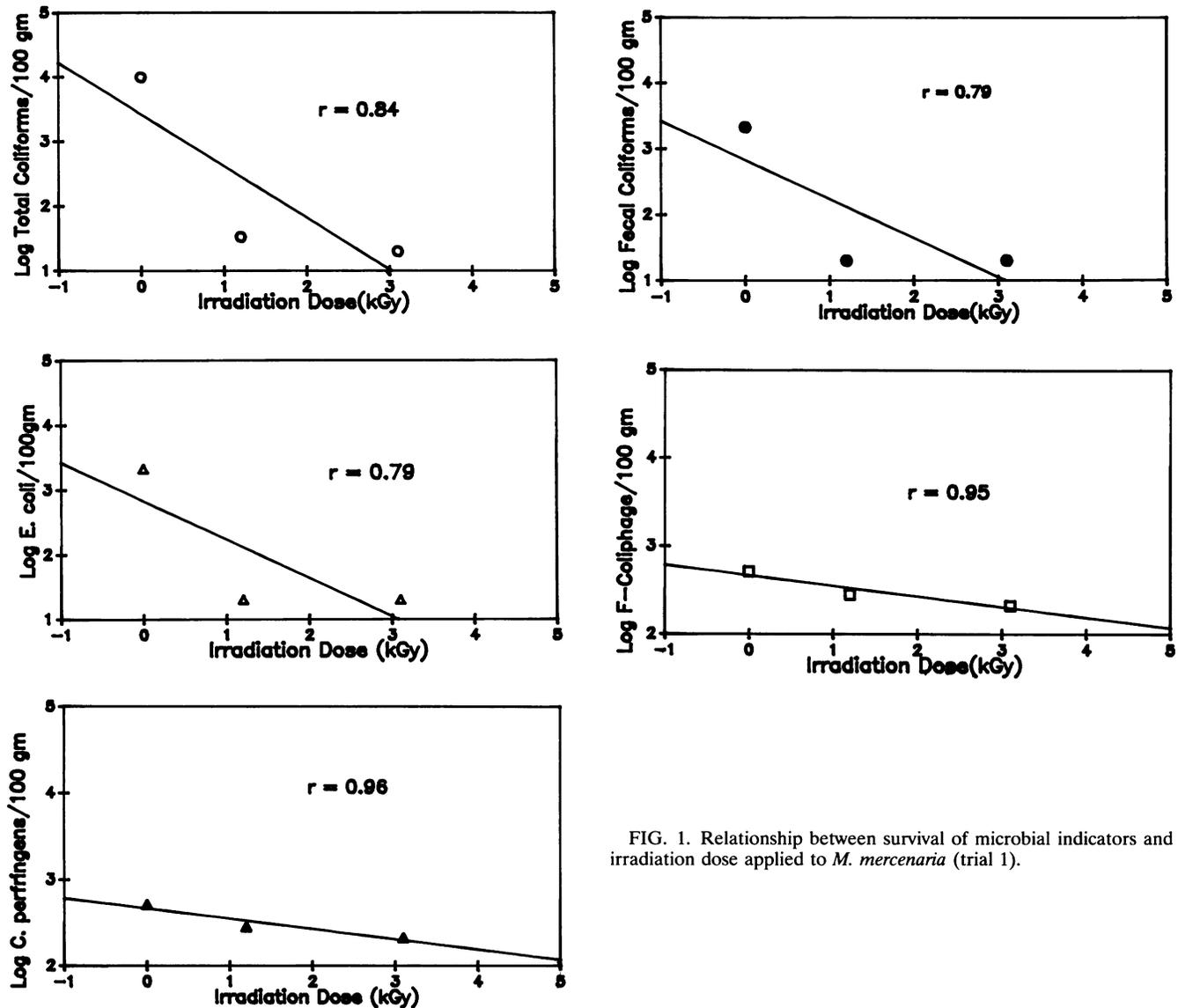
FIG. 1. Relationship between survival of microbial indicators and irradiation dose applied to *M. mercenaria* (trial 1).

which were then taped together. Irradiation exposures of 0.5, 1.5, and 3.0 kGy were targeted. Actual exposures were calculated with five dosimeters placed at the center and the four corners of the rectangular shellfish array. Controls (nonirradiated shellfish) were packaged under similar conditions and held under the same environmental conditions for the period when a corresponding batch was being irradiated.

The cobalt-60 irradiation source, which presently is rated at about 0.5 MCi, is situated in a 10-m water-shielded pool. This source is contained in aluminum cassette strips assembled in a series of racks. The samples to be irradiated were placed in an adjacent gamma exposure cave (8 by 8 ft [243.84 by 243.84 cm]). After treatment, shellfish were placed on ice in plastic bags. Microbial and viral indicator assays were begun within 24 h.

Shellfish from each irradiation treatment were randomly divided into three equal samples of 12 clams each. They were scrubbed under tap water and shucked, and the entire shell contents from each sample were placed in a sterile Waring Blendor jar. Meats and liquors were homogenized at high speed for 2 min and held on ice until analysis (within 60 min). Total coliform, fecal coliform, *E. coli*, and *Clostridium perfringens* densities were determined for each test portion by five-tube, multiple-dilution, most-probable-number procedures. Total coliform concentrations were determined according to procedures recommended by the American Public Health Association (2). EC-Mug broth (Difco Laboratories, Detroit, Mich.) was used as the confirmatory medium for fecal coliforms and *E. coli* (14). The iron milk method (1) was used to determine *C. perfringens* concentrations. F-coliphage densities were determined by a modified double-agar-overlay procedure (4).

After the radiation treatments, shellfish survival was determined daily. The hard clams were held, uncovered, at 4°C. Survival was determined by the inability of the analyst to open the shellfish when exerting light pressure on a shucking knife placed at the bill side of the junction of the valves and by the reclosing of clams (the adductor muscles were uncut) after the knife was removed.
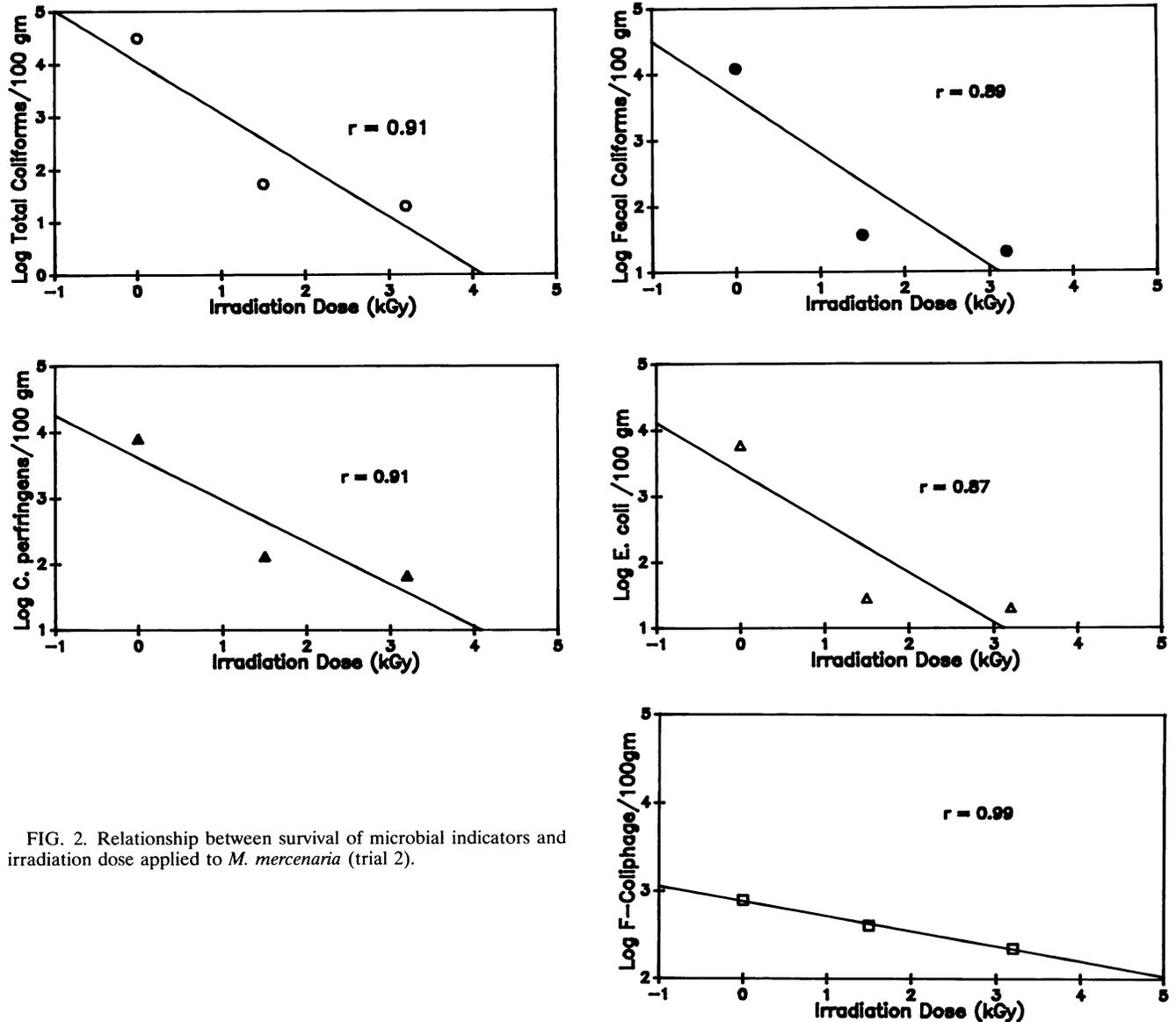
FIG. 2. Relationship between survival of microbial indicators and irradiation dose applied to *M. mercenaria* (trial 2).

Figures 1 to 3 show the relationships between the survival of bacterial and viral indicators in *M. mercenaria* and the irradiation doses applied. D_{10} values were calculated by regression analysis of the mean number of surviving microbial indicators

TABLE 1. D_{10} values of microbial indicators in irradiated *M. mercenaria*

Indicator	D_{10} values			
	Trial			Mean
	1	2	3	
Total coliforms	1.19	1.02	1.75	1.32
Fecal coliforms	1.69	1.18	1.30	1.39
E. coli	1.69	1.32	1.61	1.54
C. perfringens	4.34	1.56	2.22	2.71
F-coliphage	8.33	5.88	26.31	13.50

versus irradiation dose (kilograys). Correlation coefficients for most regression analyses exceeded 0.8. Vegetative bacterial indicator (total and fecal coliform and *E. coli*) levels appeared to be significantly reduced at exposures of ≤1.0 to 1.5 kGy (Fig. 1 and 2). The poorest regression coefficient ($r = 0.31$) observed was for phage inactivation in trial 3 (Fig. 3). This was probably a result of the relatively minimal effects of gamma irradiation on F-coliphage coupled with the significant animal-to-animal variability often found for the uptake of microbial contaminants by shellfish (3). D_{10} values were 1.02 to 1.75 kGy for total coliforms, 1.18 to 1.69 kGy for fecal coliforms, and 1.32 to 1.69 kGy for *E. coli* in live irradiated shellfish (Table 1).

The D_{10} values found in this study for *E. coli* do not agree with the lower D value ($D_{10} = 0.37$ kGy) reported earlier (9). This discrepancy may be due to differences in shellfish preparation and contamination. In this study, D_{10} values were determined from live irradiated shellfish contaminated with a
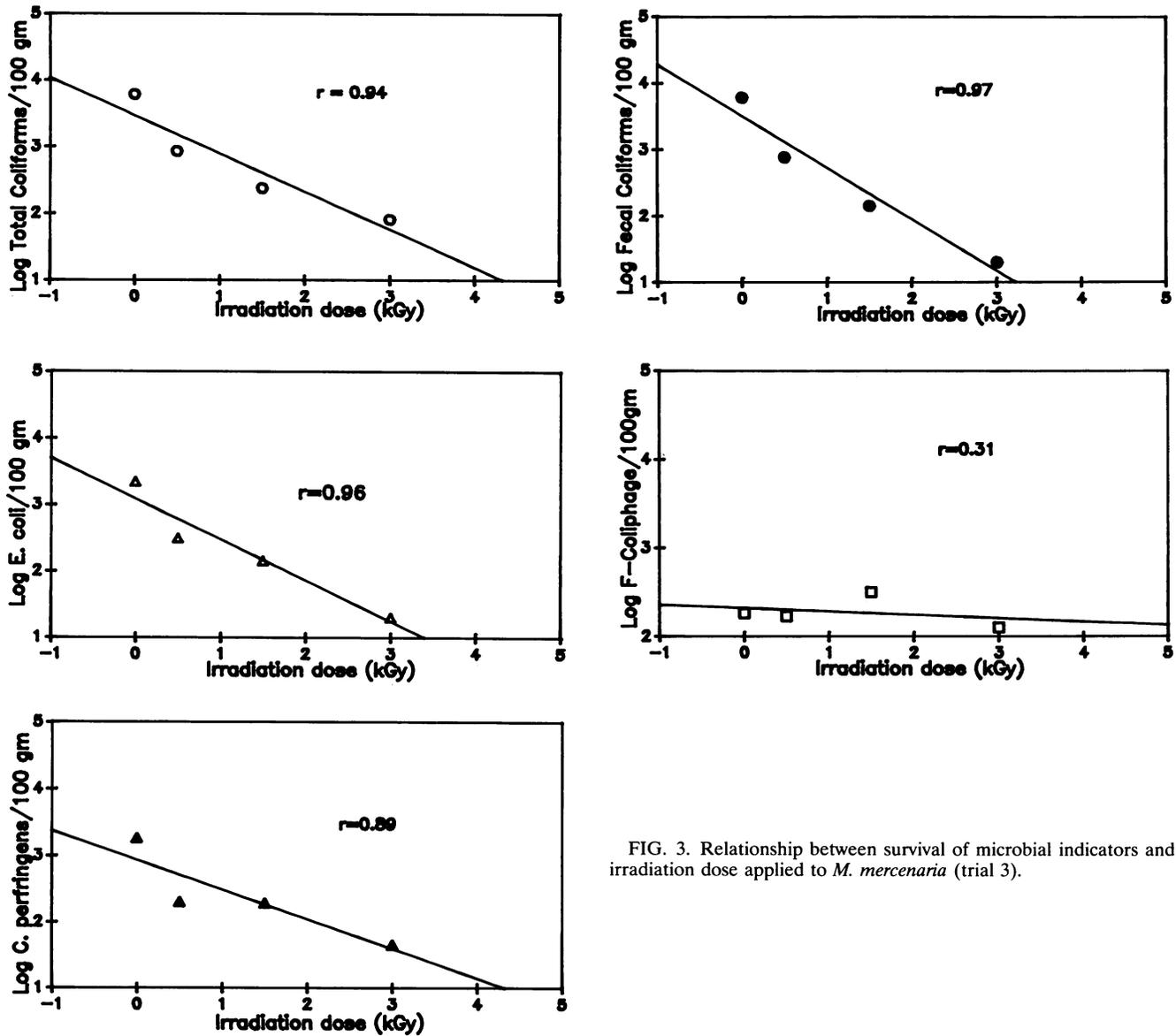
FIG. 3. Relationship between survival of microbial indicators and irradiation dose applied to *M. mercenaria* (trial 3).

natural population of indicator organisms found in wastewater, whereas the earlier study (9) determined D_{10} values from irradiated shellfish homogenate inoculated with a pure culture of *E. coli*. The present study used hard-shelled clams (*M. mercenaria*), whereas soft-shelled clams (*Mya arenaria*) were used in the earlier study (9).

C. perfringens and the F-coliphage population showed a much greater resistance to irradiation exposure and, in general, much more variability in survival at all exposures. D_{10} values ranged from 1.56 to 4.34 kGy for *C. perfringens* and from 5.88 to 26.31 kGy for the F-coliphage population.

Survival rates of shellfish after gamma irradiation are shown in Fig. 4. At exposures of ≥0.5 kGy, survival of *M. mercenaria* was very poor. No viable shellfish remained after 6 days at 1.5 kGy or after 2 days at 3.0 kGy. At 0.5 kGy, >80% of the shellfish remained viable for up to 8 days, at which time mortality rates increased significantly.

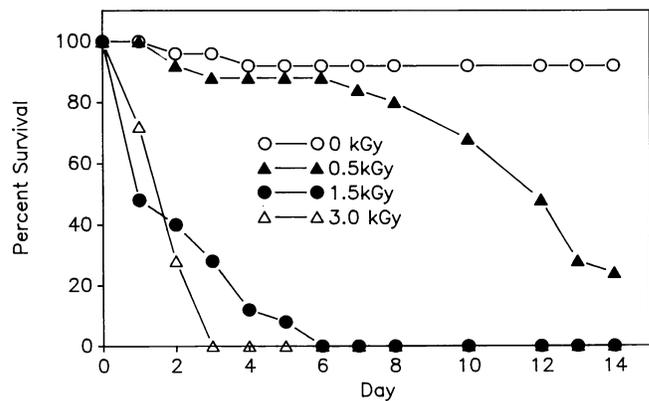After radiation exposures of ≤0.5 kGy at which prolonged

FIG. 4. Survival rates of *M. mercenaria* after exposure to gamma irradiation (trial 3).

survival of shellfish was observed (>80% survival for 8 days), vegetative bacterial densities were reduced by about 1 order of magnitude. However, the viral indicator was not significantly affected. At exposures of >0.5 kGy, shellfish mortality and inactivation of the vegetative bacterial cells were rapid, but the virus population was only minimally reduced.

These results suggest that irradiation is probably not a feasible process for inactivating viruses in contaminated shellstock and that the radiation exposures needed to significantly reduce vegetative bacterial cell populations in whole animals (e.g., hard-shelled clams) may substantially increase shellfish mortality.

REFERENCES

1. **Abeyta, C.** 1983. Comparison of the iron milk and official AOAC methods for enumeration of *Clostridium perfringens* from fresh seafoods. J. Assoc. Off. Anal. Chem. **66:**1175–1177.
2. **American Public Health Association.** 1970. Recommended procedures for the examination of sea water and shellfish, 4th ed. American Public Health Association, Washington, D.C.
3. **Burkhardt, W., III, W. D. Watkins, and S. R. Rippey.** 1992. Seasonal effects on accumulation of microbial indicator organisms by *Mercenaria mercenaria*. Appl. Environ. Microbiol. **58:**826–831.
4. **Cabelli, V. J.** 1988. Microbial indicator levels in shellfish, water and sediments from the Upper Narragansett Bay conditional shellfish-growing area. Report to the Narragansett Bay Project, Providence, R.I.
5. **Di Girolamo, R., J. Liston, and J. Matches.** 1972. Effects of irradiation on the survival of virus in West Coast oysters. Appl. Microbiol. **24:**1005–1006.
6. **Dufour, A. P., E. R. Strickland, and V. J. Cabelli.** 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. **41:**1152–1158.
7. **Josephson, E. S.** 1983. Radappertization of meat, poultry, finfish, shellfish, and special diets, p. 231–251. *In* E. S. Josephson and

M. S. Peterson (ed.), Preservation of food by ionizing radiation, vol. III. CRC Press, Boca Raton, Fla.
8. **Kilgen, M., M. Cole, and R. Grodner.** 1987. Control of indicator and pathogenic bacteria in Louisiana shellstock oysters by ionizing radiation. Abstr. Annu. Meet. Inst. Food Technol., Las Vegas, Nev., 16 to 19 June 1987.
9. **Luizzo, J. A., M. K. Farag, and A. F. Norwalk.** 1967. Effect of low level irradiation on the proteolytic activity of bacteria in oysters. J. Food Sci. **32:**104–112.
10. **Mallet, J. C.** 1991. Effect of ionizing irradiation in *Crassostrea virginica* (American oyster), p. 120–130. Activities report and minutes of work groups and sub-work groups of the R & D Associates, vol. 43. Research and Development Associates for Military Food and Packaging Systems, San Antonio, Tex.
11. **Mallett, J. C., L. E. Beghian, and T. Metcalf.** 1991. Potential of irradiation technology for improved shellfish sanitation, p. 247–252. *In* W. S. Otwell, G. E. Rodrick, and R. E. Martin (ed.), Molluscan shellfish depuration. CRC Press, Ann Arbor, Mich.
12. **Mallett, J. C., J. D. Kaylor, and J. J. Licciardello.** 1985. Depuration of bacterially contaminated live and shucked soft shelled clams *Mya arenaria*, by gamma irradiation, p. 241–242. Proceedings of the International Atomic Energy Agency/FAO International Symposium on Food Irradiation Processes, Washington, D.C.
13. **Rippey, S. R.** 1992. Shellfish borne disease outbreaks, p. 120. Northeast Seafood Laboratory, U.S. Food and Drug Administration, Davisville, R.I.
14. **Rippey, S. R., L. A. Chandler, and W. D. Watkins.** 1987. Fluorometric method for enumeration of *Escherichia coli* in molluscan shellfish. J. Food Prot. **50:**685–690.
15. **Shiflett, M. A., J. S. Lee, and R. O. Sinnhuber.** 1966. Microbial flora of irradiated Dungeness crabmeat and Pacific oysters. Appl. Microbiol. **14:**411–415.
16. **Zurer, P. S.** 1986. Food irradiation: a technology at a turning point. Chem. Eng. News **64:**46–56.