APPENDIX 4: **Bacterial Pathogen Growth and Inactivation**

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the telephone number listed on the title page of this guidance.

This appendix contains information on the growth and inactivation of bacterial pathogens.

Table A-1 contains information on the minimum water activity (a_w), acidity (pH), and temperature; the maximum, pH, water phase salt, and temperature; and oxygen requirements that will sustain growth for the bacterial pathogens that are of greatest concern in seafood processing. Data shown are the minimum or maximum values, the extreme limits reported among the references cited. These values may not apply to your processing conditions.

Table A-2 contains information on maximum, cumulative time and internal temperature combinations for exposure of fish and fishery products that, under ordinary circumstances, will be safe for the bacterial pathogens that are of greatest concern in seafood processing. These maximum, cumulative exposure times are derived from published scientific information.

Because the nature of bacterial growth is logarithmic, linear interpolation using the time and temperature guidance may not be appropriate. Furthermore, the food matrix effects bacterial growth (e.g., presence of competing microorganisms, available nutrients, growth restrictive agents). Consideration of such attributes is needed when using the information in Tables A-1 and A-2.

In summary, Table A-2 indicates that:

For raw, ready-to-eat products:

- If at any time the product is held at internal temperatures above 70°F (21.1°C), exposure time (i.e., time at internal temperatures above 50°F (10°C) but below 135ºF (57.2ºC)) should be limited to 2 hours (3 hours if *Staphylococcus aureus* (*S. aureus*) is the only pathogen of concern),

  OR

  Alternatively, exposure time (i.e., time at internal temperatures above 50°F (10°C) but below 135ºF (57.2ºC)) should be limited to 4 hours, as long as no more than 2 of those hours are between 70°F (21.1°C) and 135ºF (57.2ºC);

OR

- If at any time the product is held at internal temperatures above 50°F (10°C) but never above 70°F (21.1°C), exposure time at internal temperatures above 50°F (10°C) should be limited to 5 hours (12 hours if *S. aureus* is the only pathogen of concern);

OR

- The product is held at internal temperatures below 50°F (10°C) throughout processing,

  OR

  Alternatively, the product is held at ambient air temperatures below 50°F (10°C) throughout processing.

For cooked, ready-to-eat products:

- If at any time the product is held at internal temperatures above 80°F (26.7°C), exposure time (i.e., time at internal temperatures above 50°F (10°C) but below 135ºF (57.2ºC)) should be limited to 1 hour (3 hours if *S. aureus* is the only pathogen of concern),

  OR

Alternatively, if at any time the product is held at internal temperatures above 80°F (26.7°C), exposure time (i.e., time at internal temperatures above 50°F (10°C) but below 135°F (57.2°C)) should be limited to 4 hours, as long as no more than 1 of those hours is above 70°F (21.1°C);

OR

- If at any time the product is held at internal temperatures above 70°F (21.1°C) but never above 80°F (26.7°C), exposure time at internal temperatures above 50°F (10°C) should be limited to 2 hours (3 hours if *S. aureus* is the only pathogen of concern),

  OR

  Alternatively, if the product is never held at internal temperatures above 80°F (26.7°C), exposure times at internal temperatures above 50°F (10°C) should be limited to 4 hours, as long as no more than 2 of those hours are above 70°F (21.1°C);

OR

- If at any time the product is held at internal temperatures above 50°F (10°C) but never above 70°F (21.1°C), exposure time at internal temperatures above 50°F (10°C) should be limited to 5 hours (12 hours if *S. aureus* is the only pathogen of concern);

OR

- The product is held at internal temperatures below 50°F (10°C) throughout processing,

  OR

  Alternatively, the product is held at ambient air temperatures below 50°F (10°C) throughout processing.

Note that the preceding recommended critical limits do not address internal product temperatures between 40°F (4.4°C), the recommended maximum storage temperature for refrigerated fish and fishery products, and 50°F (10°C). That is because growth of foodborne pathogenic bacteria is very slow at these temperatures and the time necessary for significant growth is longer than would be reasonably likely to occur in most fish and fishery product processing steps. However, if you have processing steps that occur at these temperatures that approach the maximum cumulative exposure times listed in Table A-2 below for the pathogenic bacteria of concern in your product, you should consider development of a critical limit for control at these temperatures.

It is not possible to furnish recommendations for each pathogenic bacteria, process, type of fish and fishery product, and temperature or combination of temperatures. Programmable models to predict growth rates for certain pathogens associated with various foods under differing conditions have been developed by the U.S. Department of Agriculture's (Pathogen Modeling Program (PMP)) and the United Kingdom's (Food MicroModel (FMM) program). These programs can provide growth curves for selected pathogens. You indicate the conditions, such as pH, temperature, and salt concentration that you are interested in and the models provide pathogen growth predictions (e.g., growth curve, time of doubling, time of lag phase, and generation time). FDA does not endorse or require the use of such modeling programs, but recognizes that the predictive growth information they provide may be of assistance to some processors. However, you are cautioned that significant deviations between actual microbiological data in specific products and the predictions do occur, including those for the lag phase of growth. Therefore, you should validate the time and temperature limits derived from such predictive models.

Table A-3 contains information on the destruction of *Listeria monocytogenes* (*L. monocytogenes*). Lethal rate, as used in this table, is the relative lethality of 1 minute at the designated internal product temperature as compared with the lethality of 1 minute at the reference internal product temperature of 158°F (70°C) (i.e., z = 13.5°F (7.5°C)). For example, 1

minute at 145°F (63°C) is 0.117 times as lethal as 1 minute at 158°F (70°C). The times provided are the length of time at the designated internal product temperature necessary to deliver a 6D process for *L. monocytogenes*. The length of time at a particular internal product temperature needed to accomplish a six logarithm reduction in the number of *L. monocytogenes* (6D) is, in part, dependent upon the food in which it is being heated. The values in the table are generally conservative and apply to all foods. You may be able to establish a shorter process time for your food by conducting scientific thermal death time studies. Additionally, lower degrees of destruction may be acceptable in your food if supported by a scientific study of the normal initial levels in the food. It is also possible that higher levels of destruction may be necessary in some foods, if especially high initial levels are anticipated.

Table A-4 contains information on the destruction of *Clostridium botulinum* (*C. botulinum*) type B (the most heat- resistant form of non-proteolytic *C. botulinum*). Lethal rate, as used in this table, is the relative lethality of 1 minute at the designated internal product temperature as compared with the lethality of 1 minute at the reference product internal temperature of 194°F (90°C) (i.e., for temperatures less than 194°F (90°C), z = 12.6°F (7.0°C); for temperatures above 194°F (90°C), z = 18°F (10°C)). The times provided are the length of time at the designated internal product temperature necessary to deliver a 6D process for *C. botulinum*. The values in the table are generally conservative. However, these values may not be sufficient for the destruction of non-proteolytic *C. botulinum* in dungeness crabmeat because of the potential protective effect of lysozyme. You may be able to establish a shorter process time for your food by conducting scientific thermal death time studies. Additionally, lower degrees of destruction may be acceptable in your food if supported by a scientific study of the normal innoculum in the food.

TABLE A-1
LIMITING CONDITIONS FOR PATHOGEN GROWTH

PATHOGEN	MIN. A_W (USING SALT)	MIN. pH	MAX. pH	MAX. % WATER PHASE SALT	MIN. TEMP.	MAX. TEMP.	OXYGEN REQUIREMENT
BACILLUS CEREUS	0.92	4.3	9.3	10	39.2°F 4°C	131°F[1] 55°C	facultative anaerobe[4]
CAMPYLOBACTER JEJUNI	0.987	4.9	9.5	1.7	86°F 30°C	113°F 45°C	micro-aerophile[2]
CLOSTRIDIUM BOTULINUM, TYPE A, AND PROTEOLYTIC TYPES B AND F	0.935	4.6	9	10	50°F 10°C	118.4°F 48°C	anaerobe[3]
CLOSTRIDIUM BOTULINUM, TYPE E, AND NON-PROTEOLYTIC TYPES B AND F	0.97	5	9	5	37.9°F 3.3°C	113°F 45°C	anaerobe[3]
CLOSTRIDIUM PERFRINGENS	0.93	5	9	7	50°F 10°C	125.6°F 52°C	anaerobe[3]
PATHOGENIC STRAINS OF ESCHERICHIA COLI	0.95	4	10	6.5	43.7°F 6.5°C	120.9°F 49.4°C	facultative anaerobe[4]
LISTERIA MONOCYTOGENES	0.92	4.4	9.4	10	31.3°F -0.4°C	113°F 45°C	facultative anaerobe[4]
SALMONELLA SPP.	0.94	3.7	9.5	8	41.4°F 5.2°C	115.2°F 46.2°C	facultative anaerobe[4]
SHIGELLA SPP.	0.96	4.8	9.3	5.2	43°F 6.1°C	116.8°F 47.1°C	facultative anaerobe[4]
STAPHYLOCOCCUS AUREUS GROWTH	0.83	4	10	20	44.6°F 7°C	122°F 50°C	facultative anaerobe[4]
STAPHYLOCOCCUS AUREUS TOXIN FORMATION	0.85	4	9.8	10	50°F 10°C	118°F 48°C	facultative anaerobe[4]
VIBRIO CHOLERAE	0.97	5	10	6	50°F 10°C	109.4°F 43°C	facultative anaerobe[4]
VIBRIO PARAHAEMOLYTICUS	0.94	4.8	11	10	41°F 5°C	113.5°F 45.3°C	facultative anaerobe[4]
VIBRIO VULNIFICUS	0.96	5	10	5	46.4°F 8°C	109.4°F 43°C	facultative anaerobe[4]
YERSINIA ENTEROCOLITICA	0.945	4.2	10	7	29.7°F -1.3°C	107.6°F 42°C	facultative anaerobe[4]

1. Has significantly delayed growth (>24 hours) at 131°F (55°C).
2. Requires limited levels of oxygen.
3. Requires the absence of oxygen.
4. Grows either with or without oxygen.

TABLE A-2 TIME AND TEMPERATURE GUIDANCE FOR CONTROLLING PATHOGEN GROWTH AND TOXIN FORMATION IN FISH AND FISHERY PRODUCTS		
POTENTIALLY HAZARDOUS CONDITION	PRODUCT TEMPERATURE	MAXIMUM CUMULATIVE EXPOSURE TIME
GROWTH AND TOXIN FORMATION BY ***BACILLUS CEREUS***	39.2-43°F (4-6°C) 44-59°F (7-15°C) 60-70°F (16-21°C) Above 70°F (21°C)	5 days 1 day 6 hours 3 hours
GROWTH OF ***CAMPYLOBACTER JEJUNI***	86-93°F (30-34°C) Above 93°F (34°C)	48 hours 12 hours
GERMINATION, GROWTH, AND TOXIN FORMATION BY ***CLOSTRIDIUM BOTULINUM*** TYPE A, AND PROTEOLYTIC TYPES B AND F	50-70°F (10-21°C) Above 70°F (21°C)	11 hours 2 hours
GERMINATION, GROWTH, AND TOXIN FORMATION BY ***CLOSTRIDIUM BOTULINUM*** TYPE E, AND NON-PROTEOLYTIC TYPES B AND F	37.9-41°F (3.3-5°C) 42-50°F (6-10°C) 51-70°F (11-21°C) Above 70°F (21°C)	7 days 2 days 11 hours 6 hours
GROWTH OF ***CLOSTRIDIUM PERFRINGENS***	50-54°F (10-12°C) 55-57°F (13-14 °C) 58-70°F (15-21°C) Above 70°F (21°C)	21 days 1 day 6 hours[1] 2 hours
GROWTH OF PATHOGENIC STRAINS OF ***ESCHERICHIA COLI***	43.7-50°F (6.6-10°C) 51-70°F (11-21°C) Above 70°F (21°C)	2 days 5 hours 2 hours
GROWTH OF ***LISTERIA MONOCYTOGENES***	31.3-41°F (-0.4-5°C) 42-50°F (6-10°C) 51-70°F (11-21°C) 71-86°F (22-30°C) Above 86°F (30°C)	7 days 1 day 7 hours 3 hours 1 hour
GROWTH OF ***SALMONELLA*** SPECIES	41.4-50°F (5.2-10°C) 51-70°F (11-21°C) Above 70°F (21°C)	2 days 5 hours 2 hours
GROWTH OF ***SHIGELLA*** SPECIES	43-50°F (6.1-10°C) 51-70°F (11-21°C) Above 70°F (21°C)	2 days 5 hours 2 hours
GROWTH AND TOXIN FORMATION BY ***STAPHYLOCOCCUS AUREUS***	50°F (7-10°C) 51-70°F (11-21°C) Above 70°F (21°C)	14 days 12 hours[1] 3 hours
GROWTH OF ***VIBRIO CHOLERAE***	50°F (10°C) 51-70°F (11-21°C) 71-80°F (22-27°C) Above 80ºF (27ºC)	21 days 6 hours 2 hours 1 hour[2]
GROWTH OF ***VIBRIO PARAHAEMOLYTICUS***	41-50°F (5-10°C) 51-70°F (11-21°C) 71-80°F (22-27°C) Above 80ºF (27ºC)	21 days 6 hours 2 hours 1 hour[2]
GROWTH OF ***VIBRIO VULNIFICUS***	46.4-50°F (8-10°C) 51-70°F (11-21°C) 71-80°F (22-27°C) Above 80ºF (27ºC)	21 days 6 hours 2 hours 1 hour[2]
GROWTH OF ***YERSINIA ENTEROCOLITICA***	29.7-50°F (-1.3-10°C) 51-70°F (11-21°C) Above 70°F (21°C)	1 day 6 hours 2.5 hours

1. Additional data needed.
2. Applies to cooked, ready-to-eat foods only.

TABLE A-3 INACTIVATION OF LISTERIA MONOCYTOGENES			
INTERNAL PRODUCT TEMPERATURE (°F)	INTERNAL PRODUCT TEMPERATURE (°C)	LETHAL RATE	TIME FOR 6D PROCESS (MINUTES)
145	63	0.117	17.0
147	64	0.158	12.7
149	65	0.215	9.3
151	66	0.293	6.8
153	67	0.398	5.0
154	68	0.541	3.7
156	69	0.736	2.7
158	70	1.000	2.0
160	71	1.359	1.5
162	72	1.848	1.0
163	73	2.512	0.8
165	74	3.415	0.6
167	75	4.642	0.4
169	76	6.310	0.3
171	77	8.577	0.2
172	78	11.659	0.2
174	79	15.849	0.1
176	80	21.544	0.09
178	81	29.286	0.07
180	82	39.810	0.05
182	83	54.116	0.03
183	84	73.564	0.03
185	85	100.000	0.02
Note: z = 13.5°F (7.5°C).			

TABLE A-4 INACTIVATION OF NON-PROTEOLYTIC CLOSTRIDIUM BOTULINUM TYPE B			
INTERNAL PRODUCT TEMPERATURE (°F)	INTERNAL PRODUCT TEMPERATURE (°C)	LETHAL RATE*	TIME FOR 6D PROCESS (MINUTES)
185	85	0.193	51.8
187	86	0.270	37.0
189	87	0.370	27.0
190	88	0.520	19.2
192	89	0.720	13.9
194	90	1.000	10.0
196	91	1.260	7.9
198	92	1.600	6.3
199	93	2.000	5.0
201	94	2.510	4.0
203	95	3.160	3.2
205	96	3.980	2.5
207	97	5.010	2.0
208	98	6.310	1.6
210	99	7.940	1.3
212	100	10.000	1.0

Note: For temperatures less than 194°F (90°C), z = 12.6°F (7.0°C); for temperatures above 194°F (90°C), z = 18°F (10°C).

*Note: These lethal rates and process times may not be sufficient for the destruction of non-proteolytic C. botulinum in dungeness crabmeat because of the potential that substances that may be naturally present, such as lysozyme, may enable the pathogen to more easily recover from heat damage.

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We have placed the following references on display in the Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. You may see them at that location between 9 a.m. and 4 p.m., Monday through Friday. As of March 29, 2011, FDA had verified the Web site address for the references it makes available as hyperlinks from the Internet copy of this guidance, but FDA is not responsible for any subsequent changes to Non-FDA Web site references after March 29, 2011.

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