Lethality and Stabilization Performance Standards for Certain Meat and Poultry Products: Technical Paper

Food Safety and Inspection Service
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Introduction

FSIS recently made final pathogen reduction performance standards applicable to the production of certain meat and poultry products. The performance standards establish a requisite reduction of *Salmonella* (lethality) in certain ready-to-eat products and limit the growth of spore-forming bacteria of concern (stabilization) in certain ready-to-eat and partially-cooked products. To achieve the lethality performance standard, establishments are required to achieve a 7-$\log_{10}$ reduction in *Salmonella* in ready-to-eat poultry and a 6.5 -$\log_{10}$ reduction in *Salmonella* in ready-to-eat beef products. Establishments also may employ processes that achieve lower lethality reductions if they have determined that they are achieving an equivalent probability that no viable *Salmonella* organisms remain in the finished product.

This paper explains the technical considerations that were used by FSIS in defining the lethality and stabilization performance standards for ready-to-eat beef and poultry products. Simple models have been developed using data from FSIS's Nationwide Microbiological Baseline Data Collection Programs and Nationwide Federal Plant Microbiological Surveys (USDA, 1994, 1996a-f). They will be collectively referred to as "the microbiological surveys."

Lethality Performance Standards Development

The approach for defining lethality performance standards was to first define a "worst case" raw product (based on the highest measured levels of *Salmonella* in the data from the microbiological surveys), and then calculate the probability distribution for the number of surviving *Salmonella* organisms in 100 grams of finished product for various specific lethality reductions. Lethality performance standards were selected that provided low probabilities of surviving organisms for the "worst case" product. The selected probability distributions of the number of surviving organisms in 100 grams of finished product for this "worst case" product may be used to develop processes employing lethalities other than those explicitly provided in the regulations.

Identifying the "Worst Case"

To interpret the data from the microbiological surveys for establishing a "worst case," it is necessary to identify both the inherent variability of results which arises as a consequence of observing only a subset of the units of a population, and the variability of the analytical measurement procedure. To account for these two sources of variability, it is necessary to define and estimate theoretical probability distribution functions which describe the distributions of *Salmonella* density in a population of a specific meat or poultry product and of the measurement results on given samples. Specifically, let $f(x|\Theta)$ denote the distribution describing the population of sample values, where x is the unknown value in a selected sample and Θ is a vector of parameters that characterize the distribution function, f. Let $g(y|x,\sigma)$ denote the "measurement" distribution, where y is a measured result from a sample with value x, and σ is a vector describing

the measurement (analytical) variability of measurements. Further, let L denote a value such that when y is less than L then Non-Detected (ND) is reported. Then the sample likelihood function, l, of observed measured result on n samples, of which m are ND, is:

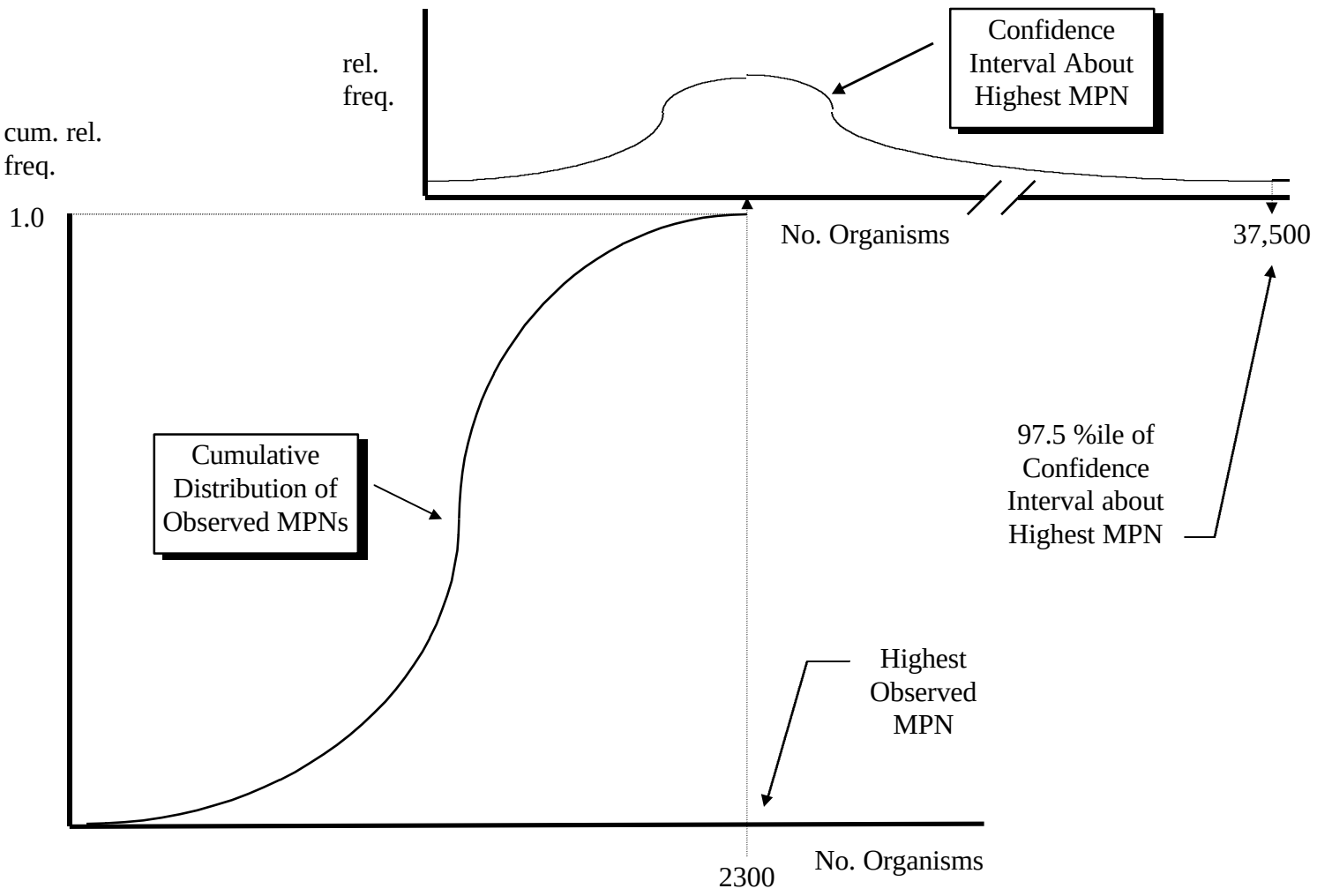
$$l = \left(\int_0^{\infty} G(L \mid x,\sigma)\, d(F(x \mid \Theta))\right)^m \prod_{i=1}^{n-m} \int_0^{\infty} g(y_i \mid x,\sigma)\, d(F(x \mid \Theta)) \qquad \textbf{(1)}$$

where $F(x|\Theta)$ be the cumulative distribution function associated with $f(x|\Theta)$ and $G(y|x,\sigma)$ is the cumulative distribution function associated with $g(y|x,\sigma)$. This likelihood equation would need to be solved in order to estimate $f(x|\Theta)$.

Estimating the distribution $f(x|\Theta)$involves extensive data analysis and assumptions about both $f(x|\Theta)$ and $g(y|x,\sigma)$. Even if $f(x|\Theta)$ was identified, decisions would need to be made concerning the percentile, and the confidence limit for this percentile to use as a demarcation value for the "worst case."

Rather than estimating $f(x|\Theta)$, a simple approach for determining a possible "worst case" was to estimate an upper confidence bound of the observed high value based on measurement error (Figure 1).

Figure 1.


rel.
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1.0
No. Organisms
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Highest MPN
Cumulative
Distribution of
Observed MPNs
Highest
Observed
MPN
2300
No. Organisms

The obtained value represents a possible value of the actual population for which only a small percent of population values would exceed. Because of sampling variability, it is possible that actual high population values would have been "missed" in a survey, particularly if there were a small number of samples. To mitigate the possible problem associated with small numbers of samples, data sets with similar statistical characteristics were combined.

The procedures for combining data entailed examining the prevalence and mean levels for *Salmonella*. Data from different products with similar expected mean levels were combined and the high value for the combined set of data was used for the different products. For this analysis, the data for ground turkey and chicken were combined and the high value for the combined data set was used for both products. As the data analysis that is presented below shows, the expected number of *Salmonella* organisms in beef products is lower than that in poultry products. Therefore, these data sets are not combined. However, all the beef data are combined and the high value for the combined data set is used for all beef products.

After establishing the initial numbers of organisms in the untreated raw product, the probability distribution of the number of surviving cells in a given processed product was determined. First, it was necessary to account for the constant changing temperature within the product. This is normally done through the use of heat transfer equations applied to the product. To determine the probability distribution of the number of surviving organisms, it is necessary to mathematically integrate probabilities. However, FSIS did not have sufficient information to determine the heat transfer equations for all individual products and to make the calculations. Thus, in calculating survival probabilities, a conservative approach was taken by assuming that the probability of surviving *Salmonella* is constant throughout the product. Then a binomial distribution was used to determine the probability distribution of the number of surviving *Salmonella*.

Therefore, starting from a high observed value from the microbiological surveys, the procedure used for identifying the "worst case" and computing the probabilities of the number of surviving organisms in portions of product can be summarized in four steps:

1) FSIS computed a 97.5% upper confidence bound, due to measurement error, for the high value. The *Salmonella* densities were obtained through a Most Probable Number (MPN) determination. The MPN procedure is a widely used standard microbiological technique for obtaining quantitative estimates of bacteria. It is described as a "multiple tube dilution to extinction method," where replicate culture tubes are set up with several dilutions of sample to the point where there are no viable organisms present. This procedure applies the theory of probability to the determination of a microbial population. The "most probable number" of viable target bacteria in a sample can be estimated from the pattern of positive tube results using the appropriate statistical probability tables.[1, 2, 3]

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[1] Oblinger, J. L., and Koburger, J. A. (1975) Journal of Milk Food Technology 38:540-545.

[2] Peeler, J. T., et al. (1992) The Most Probable Number Technique. In: Compendium of Methods for the Microbiological Examination of Foods (3rd Edition), pp. 105-120.

[3] Harrison, M. A., et al. Food Microbiology Lab Manual (Department of Food Science and Technology, University of Georgia) Athens,

The 97.5% upper confidence bound estimate is based on an approximation which results in an estimated value that is probably slightly higher (positive bias) than the true 97.5% percentile and an assumption of the analytical false negative rate. The actual calculation procedure is described in the next section. The 97.5% was chosen to represent boundaries for the "worst case" as a compromise between using a higher percentile which might identify an unrealistic high level for an already conservative choice of using the highest observed sample value over combined data sets, versus using a lower percentile (such as 90%) which might identify a value which might too often be exceeded. The use of a high confidence level also helps assure that the calculated "worst case" density represents a high percentile of the distribution of values in the populations.

2) The calculations addressed the possibility of non-recovery of organisms in samples. Based on FSIS experience with inoculated quality assurance samples, we have had repeated success in recovering 0.5 salmonellae cells per 25-gram from previously frozen samples. Thus we make the assumption that there is 99% probability that a 25-gram sample with 13 cells would test positive. Even if one organism is recovered, then the sample result would be positive, so that the probability of a positive sample result can be expressed as $1-\tau^{13}$, where τ is the theoretical probability of a single injured or uninjured *Salmonella* organism not being recovered. With this assumption, for frozen samples, τ is approximately 70%, or a 30% recovery of organisms. For non-frozen samples, the recovery rate is assumed to be doubled, to 60%.

3) For computing the probabilities of the number of surviving cells in 100 grams of cooked product, the value obtained in step 2 is multiplied by 100/0.7 = 143 grams. The 0.7 divisor accounts for a 70% yield upon cooking, which comes from FSIS assumptions used for comparing equivalent fresh and cooked product weights. Since only 25 grams of product were actually analyzed, the assumption that the high obtained value from the survey represents the density throughout the product probably overestimates the actual density. This is because maximum densities for small volume samples tend to be greater than the average density for a larger sample size.

4) FSIS used a binomial distribution with probability of survival equal to 1/(10 raised to the log lethality). This assumption is derived from the standard theory of stochastic processes assuming a simple (first order kinetic) death process (Bharucha-Reid, 1960). The critical assumptions are that the death events are independent among organisms, the distribution for the number of organisms surviving a lethality process is binomial, and the specified probability of an organism surviving is independent of the initial number of organisms. Studies that are used to estimate the probability of pathogens surviving cooking use high numbers of organisms that are inoculated into raw product.

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Measurement Properties of MPN

Calculation of MPN is based on a maximum likelihood determination from the observed pattern of positive results from the 3-tube/3-dilution analyzes. Assume a sample of meat has N organisms. If T represents the total volume of sample, and V represents a volume of a subsample for a given tube, then the probability that the subsample has no organisms is approximately: $P_0=(1-V/T)^N$. As T approaches infinity, such that the density, N/T, is a constant, r, P_0 can be approximated as $P_0=e^{-rV}$. Using this approximation, the value of r that maximizes the likelihood function is the MPN. [4]

To account for possible false negatives, it will be assumed the false negative rate depends upon the number of organisms in the subsample of volume V. The number of organisms found in a subsample is approximated by a Poisson distribution with parameter rV. Let τ represent the probability of a negative result given one organism in the sample. It will be assumed that the probability of a negative finding on a subsample with k organisms τ^k. Then, the probability of a negative result on a subsample is:

$$P_0(V,r,\tau) = e^{-rV} + e^{-rV}\sum_{i=1}^{infinity}(rV\tau)^i/i! \qquad \textbf{(2)}$$
$$= e^{-rV} - e^{-rV}(1-e^{rV\tau}) = e^{-rV(1-\tau)}$$

using the equality, $e^x = \sum x^i/i!$. Let V_j, j=1,2,3 be the three volumes of the tubes that are used for determining the MPN value, and let x_j, j=1,2,3 be the number of positive tubes from among the three tubes of volume V_j. On the standard MPN table, a MPN density value, mpn(x) is determined for each three-tube result, $x= \{x_1, x_2, x_3\}$, through a maximum likelihood calculation where it is assumed that the false negative rate , τ, is zero. Designate $P_0(V_j,r, \tau)$ to be the probability of a negative result on a tube with

[4] Cochran, William G. (1950) Estimation of Bacterial Densities by Means of the Most Probable Number. Biometrics June:105-116.

volume V_j assuming a true density of r and a false negative rate for a single organism, τ. Then, using the binomial distribution, the probability, p(x) of the obtaining result x, and thus of obtaining the MPN value of mpn(x), is given in equation 3 as:

$$p(x_1, x_2, x_3, \tau) = \prod_{j=1}^{3} \binom{3}{x_j} (1 - P_0(V_j, r, \tau))^{x_j} P_0(V_j, r, \tau)^{3-x_j} \qquad \textbf{(3)}$$

From equation 3 the cumulative distribution function of MPN results can be determined. The effect that τ has on the probabilities of MPN results can be seen in Table 1, which contains the cumulative distribution of MPN measurements as a function of the density of the sample and the false negative rates, (0% and 70%), for a subsample with one organism.

From Table 1 it can be seen that, for a sample with a density of 125 organisms/g, when the false negative rate for a tube is 0.70^k where k is the number of organisms in a tube, the probability that a MPN result is less than 10/g is 2 percent. If it is assumed that the false negative rate is zero, then the probability is approximately 0%.

A 1-α upper confidence bound, u_α, for the true unknown density of a sample with a measured MPN value, x, is defined as a value for which the probability of obtaining an MPN of x or less equals α. For example, if x = 2300 MPN, and τ = 0.70, then an upper 97.6% confidence bound is approximately 37,500 organisms per gram. In other words, if the true density were 37,500 per gram, then there is a 2.4% probability that the MPN result would be 2300/g or less.

The MPN determinations were performed using a sequence of dilutions, until both negative and positive results were obtained. The MPN readings were then taken from a 3-tube, 3-dilution table. The above calculation for the confidence interval serves as a conservative estimate of the actual confidence interval.

Table 1: Cumulative Distribution (%) of 3 Tube/3 Dilution MPN Measurement With Volumes of 0.1, 0.01, and 0.001 ml for Density $\leq$ 125/g , and of 0.01,0.001, and 0.0001 for Density > 125/g as Function of Sample Density of Organism per Gram (Org/g) And False Negative Rate, F^k, For Tube With K Organisms.

	50 ORG/g		125 ORG/g		500 ORG/g		1000 ORG/g	
False Negative Rate (F)	0%	70%	0%	70%	0%	70%	0%	70%
MPN value	Percentiles (%) of Cumulative Distribution							
ND	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.000
$\leq$10	0.39	36.6	0.00	2.08	0.00	0.00	0.00	0.000
$\leq$20	1.2	50.4	0.00	5.01	0.00	0.00	0.00	0.005
$\leq$35	20.8	81.7	1.62	33.9	0.00	1.04	0.00	0.01
$\leq$50	60.3	96.9	14.3	73.9	0.00	8.11	0.00	0.27
$\leq$100	89.9	99.8	49.3	95	0.39	36.6	0.00	5.59
$\leq$200	93.8	100	62.0	97.1	1.20	50.4	0.00	11.5
$\leq$240	99.2	100	88.6	99.7	20.6	81.5	3.70	45.6
=All[1]	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.0
mean[2]	70.6	21.5	183	53.9	705.9 842	214.9	1430	436
CV(%))))	94.6	89.2	106	91.1	94.6	89.2	92.3	103.4

[1] All: Probability that all 9 tubes are positive.

[2] ND (all tubes negative) was assigned a value of 1/g, and a value of 4800/g was assigned if all tubes were positive.

Considering these “upper confidence bounds” and the corresponding percentiles as describing a continuous distribution, a “best” fit continuous distribution was found for approximating the percentiles of the upper confidence bounds when a measurement of approximately 2300 (between

2300-2400) MPN was obtained. The distributions tried are those that are offered within the package *BestFit Probability Distribution Fitting for Windows*®. Using the Kolmogorov-Smirnov test statistic, the selected distribution was a lognormal distribution, with mean of the natural logarithmic values equal to 9.16 and standard deviation of the natural logarithmic values equal to 0.69. The comparison of exact calculations of percentiles and estimated percentiles using the lognormal distribution is given in Table 2.

Table 2: Comparisons of Upper Confidence Bounds Using Exact Calculation and Fitted Lognormal (9.16, 0.69) for Selected Percentiles, for Measured MPN of 2300.

Upper Bound	2200	3700	6200	8700	12200	20200	42700
Exact Calculated Percentile	2.02	8.59	25.9	43.4	65.2	86.0	98.5
Fitted Lognormal Percentile	1.68	8.53	26.7	44.8	64.1	86.2	98.5

Selected statistics from the FSIS microbiological surveys are provided in Table 3. For poultry carcasses, the measured MPN per ml of rinse was converted to MPN/cm^2 using the relationship between the weight of the bird and its surface area (i.e., SA = 0.87 weight(grams) + 635). [5] Thus, for example, a 1500 gram carcass would have approximately 1940 cm^2. For beef carcasses, 3 samples per carcass were excised (each with a surface area of 300 cm^2 and a thickness of 1 cm) and combined. A "representative" subsample from the composite of the 3 samples was analyzed for MPN. The MPN results were reported as a density per cm^2. For all ground product samples, MPN counts were determined and expressed per gram of product.

Direct comparison of the levels of organisms cannot be made for poultry and beef carcasses because of the different procedures of sampling and measuring MPN. An examination of the results for ground product can be used to lead to the conclusion that for similar size cuts of whole poultry and beef portions there would be higher prevalence and/or higher levels of *Salmonella* on the poultry portions.

[5] Thomas, N. L. (1978) Observation of the relationship Between the Surface Area and Weight of Eviscerated Carcasses of Chickens, Ducks, and Turkeys. Journal of Food Technology 13:81-86.

From Table 3, it can be seen that the percentage of samples that were positive in the quantitative test is larger for the ground poultry survey than for ground beef samples. The mean values that are given in Table 3 do not include the MPN non-detectable (ND) results. Thus, the mean levels reported in Table 3, overstate the *Salmonella* levels more for beef products than for poultry products. In spite of this bias, the average MPN *Salmonella* levels for both ground chicken and turkey are more than 20 times higher than that for ground beef. Thus, *Salmonella* prevalence and levels are clearly higher in ground poultry than in ground beef. The relatively higher prevalence and levels of *Salmonella* in ground poultry indicate that higher prevalence and levels would be expected in similar size whole cuts of poultry compared to beef.

Table 3: Geometric Mean Values of MPN[1] Values and High MPN Values For *Salmonella* Positive Samples.

National Baseline Studies	Number of Samples	Number Analyzed for MPN (Number of *Salmonella* Qualitative Positive)	Number MPN Posi-Tive	Geometric Mean of MPN Positive Samples	Range[2] of Geom. Mean	High MPN
Steers & Heifers	2089	19	4	.12 MPN/cm^2	(.03, .40) MPN/cm^2	0.23 MPN/ cm^2
Cows & Bulls	2112	53	21	.27 MPN/cm^2	(.05, 1.4) MPN/cm^2	240 MPN/ cm^2
Market Hogs	2112	169	77	.22 MPN/cm^2	(.12, .42) MPN/cm^2	23 MPN/ cm^2
Broiler Chicken	1297	260	151	.033 MPN/cm^2	(.025, .043) MPN/cm^2	66 MPN/ cm^2
Raw Ground Chicken	285	131	76	1.26 MPN/g	(1.17, 1.35) MPN/g	2300 MPN/ g
Raw Ground Turkey	296	95	32	2.63 MPN/g	(1.23, 5.62) MPN/g	46 MPN/ g
Raw Ground Beef	563	29	8	.05[3] MPN/g	(.0001, 23.99) MPN/g	>110 MPN/ g

[1] Geometric mean of positive MPN values.

[2] Range computed using 3 times the standard error of the mean $\log_{10}$ MPN values.

[3] Highest MPN obtained was greater than 110 MPN/g. A value of 240 MPN/g was used in calculations.

Discussion of Poultry Results

The results for ground chicken and turkey are similar, so separate lethality requirements are not warranted. From each sample that had a qualitatively positive result for *Salmonella*, a frozen reserve subsample was quantitatively analyzed for MPN. The highest MPN value for the ground poultry products was 2300 MPN/g.

Based on 581 poultry samples and assuming a Poisson distribution, the percentage of results that would correspond to the high value of 2300 MPN/g value in a theoretical population of ground poultry samples could range, with 99% confidence, from 0.00086% to 1.279%. That is to say, it is possible that approximately 1% of 25-gram portions of ground poultry could have MPN values of about 2300 MPN/g or higher.

As mentioned above, there is measurement variability associated with the MPN determinations. For a single 2300 MPN/g determination, the 97.5% upper confidence bound is approximately 37,500 organisms per gram, assuming a 30% recovery rate.. This value serves as a basis for defining the "worst case."

For evaluating the possible number of organisms that could survive and thus present a risk to consumers, it is assumed that a consumer is eating a 100 ($\approx$3.5 ounces) grams of ready-to-eat ground poultry product. Further, it is assumed that there is a 70% yield after cooking. Therefore, 100 grams of ready-to-eat product is equivalent to 143 grams of raw product. For 143 grams of raw product, a 97.5% confidence upper limit for the number of *Salmonella* in the product is approximately 5,362,500 total organisms. Thus, for the "worst case" the number of organisms in 143 grams of raw ground poultry product is assumed to be 6.7 $\log_{10}$.

For whole poultry carcasses, the high value was 66 *Salmonella* MPN/cm^2. The 97.5% upper confidence bound of this value is approximately 700 MPN/cm^2, assuming a 30% recovery of *Salmonella* in the actual rinse solution. Assuming a carcass weight of 1500 grams, or approximately 1940 cm^2, the 97.5% upper bound of the total number of organisms on the carcass would be 6.13 $\log_{10}$ or approximately 900 per gram. The actual numbers of *Salmonella* on a carcass could be greater because not all *Salmonella* on a carcass is transferred to the rinse solution and recovered in the microbiological analysis. Let r be the percent of organisms that are transferred to the rinse and are recovered. If we assume, for example, that any one of the possible 143 - gram servings from the carcass would contain a density twice the average density, then to reach a "worst case" density of 6.7 $\log_{10}$ per 143 grams the value of r would need to be equal to approximately 5%. To determine the actual range of the number of *Salmonella* is not possible at this time because the transfer and recovery rate of the rinse procedure is unknown; however, by the above calculation, if the rate is greater than 5% and the degree of heterogeneity of the distribution of organisms on a carcass is not "too" great, then the performance standard developed to provide a safe product for the "worst case" of 6.7 $\log_{10}$ per 143 grams (which was derived from density measurements of ground poultry) should also provide a safe product for product derived from poultry carcasses.

Discussion of Beef Results

The high MPN value for *Salmonella* in ground beef products was >110/g. The result means that all of the 9 MPN tubes were positive. Thus, it is impossible to determine what the actual level might be because there is no end point. Therefore, FSIS assigned a theoretical value of 240 MPN for this result in all subsequent calculations. If the true density were 240 organisms per gram, then for a perfect MPN test using sample volumes of 10, 1, and 0.1 ml, with false negative rate equal to 0, there is a 75% probability that all 9 tubes would be positive and the result would be recorded as >110 MPN/g; if the false negative rate per tube were 0.70^k where k is the actual number of organisms in the tube, then there is approximately a 13% probability that all of the tubes would be positive. If the true density were 720 organisms per gram, then there is a probability of approximately 70% that all of the tubes would be positive.

For beef carcasses, the high *Salmonella* MPN value was an actual measured 240/cm^2. Based on 4200 samples (combining the steers and heifers survey with the cows and bulls survey) and assuming a Poisson distribution, the percentage of results that would correspond to the 240 MPN/cm^2 value in a theoretical population of carcass samples could range, with 99% confidence, from 0.00012% to 0.18%. That is to say, it is possible that approximately 0.2% of 900 cm^2 samples could have MPN values of about 240 MPN/cm^2 or greater. The selected samples were composited from 3 sections of carcass, so that, in actuality, the levels of high MPN values on contiguous sections could be higher than stated here. Because the prevalence for beef was relatively low (approximately 1-2%), it is quite possible that only one subsample from the 3 would be positive. Thus, the density for that one positive subsample would be actually 3 times 240. Thus, for beef carcasses a high value of 720/ cm^2 is assumed.

If it is assumed that the bacteria are primarily on the surface, then the density of organisms per gram of product would depend upon the thickness of the cut of meat used. It is assumed that the cut of meat is 0.8 cm and that the specific density of beef is approximately 1.1 grams/cm^3 (slightly lower than average). These factors are for practical purposes equal to 1, so that the MPN/cm^2 are assumed to estimate the density per gram of product. Thus 720 MPN/cm^2, which is used for defining the "worst case," therefore represents 720 organisms per gram, which is approximately a ½ $\log_{10}$ below that of ground poultry. Hence the "worst case" for whole beef cuts is assumed to be 6.2 $\log_{10}$ per 143 grams of raw product.

Estimates of Probabilities of Surviving Numbers of *Salmonella*

Once the number of organisms in raw product is determined, it is possible to estimate the probabilities of the number of surviving organisms for a given x-$\log_{10}$ lethality reduction process. As discussed above, under the previously stated statistical assumptions, the distribution of surviving organisms is a binomial distribution. That is, for a lethality process with a x-$\log_{10}$ reduction, the probability, p, of any given organism surviving is $p = 10^{-x}$, and the distribution of the number of organisms surviving, given N organisms in the untreated raw product, is a binomial distribution with parameters p and N.

As stated in the introduction, under new FSIS regulations, establishments are required to achieve a 7-$\log_{10}$ reduction in *Salmonella* in ready-to-eat poultry and a 6.5 -$\log_{10}$ reduction in *Salmonella* in ready-to-eat beef products. Establishments also may employ processes that achieve lower lethality reductions if they have determined that they are achieving an equivalent probability that no viable *Salmonella* organisms remain in the finished product. The probability distribution of the number of surviving organisms as a function of the number of organisms in the raw product and expected log reductions of 6.5 and 7 are given in Tables 4a and 4b, respectively.

Table 4a: Probability Distribution of Surviving Organisms in Finished Product After a 6.5-Log_{10} Lethality Reduction.

log Number of Organisms in Raw Product	Probability of Surviving Organisms (%) > 0 Surviving	> 1 Surviving	> 2 Surviving	> 3 Surviving	> 4 Surviving
6.0	27.1107	4.0610	0.4166	0.0324	0.0020
6.2	39.4189	9.0564	1.4478	0.1767	0.0174
6.5	63.2121	26.4241	8.0301	1.8988	0.3660
6.7	79.5030	47.0175	21.2745	7.6745	2.2859
7.0	95.7671	82.3814	61.2168	38.9073	21.2702

Table 4b: Probability Distribution of Surviving Organism in Finished Product After a 7- Log_{10} Lethality Reduction.

log Number of Organisms in Raw Product	Probability of Surviving Organisms (%) > 0 Surviving	> 1 Surviving	> 2 Surviving	> 3 Surviving	> 4 Surviving
6.0	9.5163	0.4679	0.0155	0.0004	0.0000
6.2	14.6568	1.1308	0.0589	0.0023	0.0001
6.5	27.1107	4.0610	0.4166	0.0324	0.0020
6.7	39.4189	9.0564	1.4478	0.1767	0.0174
7.0	63.2121	26.4241	8.0301	1.8988	0.3660

For comparing the effects of different lethality reductions, an examination of the probabilities of more than 4 organisms surviving is made. For a given "worst case" product and lethality treatment, this probability, should be very low. It can be seen from Table 4a that a 6.5-$\log_{10}$ lethality reduction in the specific case of 6.7 $\log_{10}$ number of organisms in the raw product (that is the aforementioned "worst case" for ground poultry) indicates that there is an approximate 2.3% probability that more than 4 organisms will survive the lethality process. However, with a 7-$\log_{10}$ reduction the probability of more than 4 surviving *Salmonella* is 0.0174%, or an expected once in every 5,750 times.

Similarly, in the case of an initial 6.2 $\log_{10}$ number of organisms in the raw product (that is the aforementioned "worst case" for beef products), a 6.5 $\log_{10}$ lethality reduction is required to achieve a probability of 0.0174% that greater than 4 organisms survived.

The probability distribution of the number of surviving organisms defined by the entries in the rows of Tables 4a and 4b can be used to develop alternative lethalities. Table 5 defines the probability distribution curve of the number of surviving organisms in 100 grams of finished product after a lethality treatment of 7 $\log_{10}$, assuming there were 6.7 $\log_{10}$ organisms in the pre-processed product.

Table 5: Probability of More than Specified Number of Surviving *Salmonella* per 100 Grams of Finished "Worst Case" Product.

Specified Number of Surviving *Salmonella* for Given "Worst Case" Product	>0	>1	>2	>3	>4
Probability of More than Specified Number of *Salmonella* Surviving	39.42%	9.06%	1.450%	0.1760%	0.0174%

Specifying Lethality Performance Standards and Their Equivalents

As a result of the above considerations, the lethality performance standards are being established as a 7-$\log_{10}$ lethality reduction for poultry products and a 6.5-$\log_{10}$ reduction for whole cut beef products. From the above consideration, an equivalent lethality is defined in terms of the probability distribution described in Table 5. Thus, the lethality performance standard would be satisfied if it could be demonstrated that for a theoretical "worst case" product (when there are 6.7 $\log_{10}$ per 143 grams of *Salmonella* in raw poultry or 6.2 $\log_{10}$ per 143 grams of *Salmonella* in whole muscle beef) the probability distribution of the number of surviving *Salmonella* following lethality treatment is "below" that given in Table 5. That is, the probabilities of more than a specified number of surviving organisms can not be greater than those probabilities given in Table 5.

Stabilization (Cooling)

After the product is cooked, heat-shocked spores of such microorganisms as *Clostridium botulinum* and *Clostridium perfringens* can germinate, becoming vegetative cells that can multiply to hazardous levels if cooling is inadequate. Viable counts of 10^5 or greater of *Clostridium perfringens*/gram have been recommended by the U.S. Centers for Disease Control and Prevention as one of the criteria for incriminating *Clostridium perfringens* as the causative agent of foodborne illness in finished product (CDC, 1996). However, at least 10^6 or more *Clostridium*

perfringens per gram are usually found in foods implicated in outbreaks. [6, 7] FSIS considered both of these values in developing the performance standard for stabilization: "There can be no multiplication of toxigenic microorganisms such as *Clostridium botulinum*, and no more than a 1 $\log_{10}$ multiplication of *Clostridium perfringens* within the product."

Data from the FSIS microbiological surveys indicate a "worst case" of approximately 10^4 (4 $\log_{10}$) per gram density of *Clostridium perfringens* on the raw product. For raw beef carcasses, there were 5 out of 4191 samples analyzed (0.12%) with results that were greater than 10^4 but less than 10^5 CFU/cm^2; there were 17 (0.41%) results greater than 10^3 but less than 10^4 CFU/cm^2. A CFU/cm^2 density measurement on beef approximates a density per gram measurement (see the microbiological surveys). For the ground product surveys, establishments were not selected with probability proportional to production volume. Therefore, it was necessary to determine the distribution of the densities to weight the sample results, taking into account the probability of selection, the volume of the establishments, and the non-response. Table 6 provides the estimated distribution of *Clostridium perfringens* per gram estimated from the ground product surveys.

Table 6: Product-Specific Distribution of Density[a] of *Clostridium perfringens* (CFU per gram) From FSIS Raw Ground Product Surveys.

	Gr. Beef	Gr. Chicken	Gr. Turkey	Gr. Pork
Number of Samples	563	285	296	543
ND[b]	46.7%	49.4%	71.9%	85.1%
≤ 10/g	49.8%	62.7%	79.0%	85.7%
≤ 100/g	87.7%	94.3%	94.9%	99.7%
≤ 500/g	99.4%	99.4%	96.2%	99.86%
≤ 1000/g	99.5%	99.7%	97.9%	99.95%
Maximum value	4000 CFU/g	11,000 CFU/g	3500 CFU/g	3300 CFU/g

[6] Hauschild, A. (1975) Criteria and Procedures for Implicating Clostridium Perfringens in Food-borne Outbreaks. Canadian Journal of Public Health 66:388-392.

[7] McClane, B.A. (1992) Clostridium Perfringens Enterotoxin: Structure, Action, and Detection. Journal of Food Safety 12:237-252.

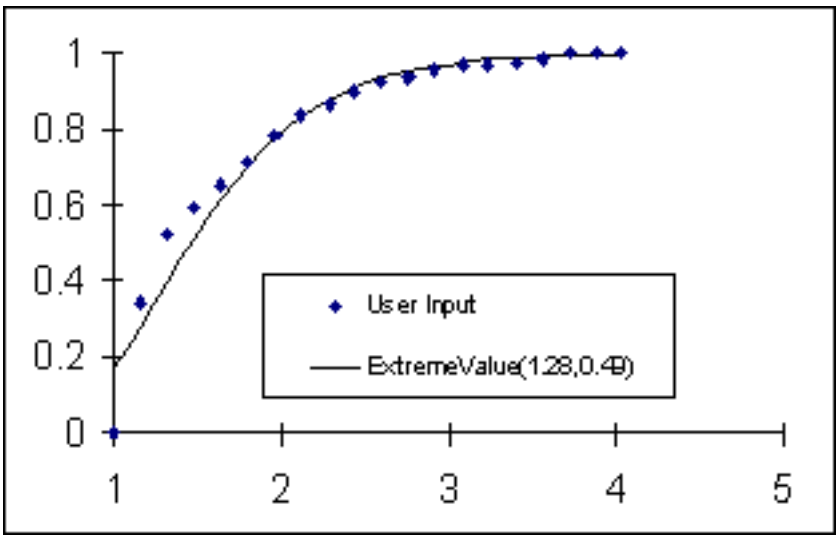
[a] Sample results were weighted by inverse probability of establishment selection, an adjustment for non-response, and an estimate of establishment production.
[b] ND indicates that in a 1 ml subsample of a 1:10 dilution no CFU was found. If there were 10 CFU per gram in the 25 gram ground sample, then (using the Poisson distribution) there would be a 36.8% chance of a ND finding.

A very small percentage of samples described in Table 6 have densities more than 1000 CFU/g. One sample had estimated density of more than 10^4 CFU/g. Moreover, as described in Table 6, the distribution of these densities is highly skewed. The sample cumulative distribution of the common logarithm of positive CFU/g findings (unweighted) versus that of an estimated extreme value distribution: F(x) = exp-(exp(-(x-1.28)/0.49)) is presented in Figure2. This distribution was derived using the program: ***BestFit*** *Probability Distribution Fitting For Windows*® version 2.0c. The percentage of the samples that are positive vary by product (Table 6) ranging from about 15% to 53%. Designating α to be the fraction of the samples that are positive, then, for large densities, it can be estimated that the probability of a randomly selected sample, with density, d, being greater than a given value, d_0, is:

$$Prob(d > d_0 \mid a) = (1 - a)(1 - \exp(\exp(-(\log_{10}(d_0) 1.28)/0.49))) \qquad \textbf{(4)}$$

For example, if $\alpha = 0.6$, then the probability that a result would exceed 10^4 CFU/g would be 0.155%, or approximately once in 645 samples. If $\alpha = 0.7$, then the probability is 0.116, or once in every 846 samples.

Figure 2: Distribution of Common Logarithm of Positive CFU/g Compared With Extreme Value Distribution: F(x) = exp(-exp(-(x-1.28)/0.49))



The results from the carcasses and ground product surveys indicate that small percentages of samples may have densities above 10^4 organisms per gram., and that it would be unlikely that any significant number of samples would have densities above 10^5 organisms per gram. If cooling results in a 1 $\log_{10}$ relative growth of *Clostridium perfringens*, then there would be only a small percentage of samples with more than 5 $\log_{10}$ per gram density of *Clostridium perfringens* in the final product, but a non-significant number of samples with 6 $\log_{10}$ per gram density or more. Consequently, FSIS is requiring that cooling processes that are used by establishment shall result in less than a theoretical 1 $\log_{10}$ relative growth of *Clostridium perfringens*.

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