ENVIROTECH

HB2

EFFICACY REPORT

Data Summary: Efficacy of Several Antimicrobial Processing Aids Used on Meat, Pork, and Poultry Against E. coli 0157:H7 and Salmonella for FCN #944: Supplementary Addendum

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Background

Enviro Tech Chemical Services, Inc. applied for the submission of a food-contact substance notification (FCN 000944) on October 9, 2009 for an antimicrobial water treatment (processing aid) for use on meat and poultry as a general water treatment compound. The FCS is hypobromous acid, which is blended on site by mixing hydrogen bromide and a hypochlorite (such as sodium hypochlorite) to a water source. A letter in response to Enviro Tech Chemical Services, Inc. FCN 000944 was received November 23, 2009 and stated that The Department of Health and Human Services requested additional data demonstrating statistical differences or variability within test groups of microbial testing performed for these studies and use instructions of the FCS. The study is being submitted before the requested deadline of December 8, 2009.

Therefore, this is a supplementary addendum to the original submission and was compiled in response to the above mentioned correspondence in which The Department of Health and Human Services requested additional data. This addendum includes statistical analysis of the efficacy data for meat (Attachment #7 of the FCN titled "Efficacy of Several Antimicrobial Processing Aids Sprayed on Meat and Pork Products Against E. coli 0157) and efficacy data for poultry (Attachment #8 of the FCN titled "Efficacy of Several Antimicrobial Processing Aids On Poultry Using Spray and Submersion Methods Against Salmonella"). This submission also includes a separate letter addressing comments on use instruction of the FCS.

Applicant's comments: This applicant acknowledges that study Attachments #7 and #8 are not broad definitive reports of the efficacy of any one particular FCS. Our intent was to show a relative comparison of established, proven, and commonly-used food contact antimicrobials under the harshest most severe conditions we could reasonably perform.

DBDMH has been evaluated by the FDA and FSIS numerous times, which have resulted in the inclusion of the antimicrobial in the FSIS Directive List 7120.1, and have become FDA FCN's as follows:

FCN #334: DBDMH approved for use in poultry chiller water as an antimicrobial at 100 ppm.

FCN #357: DBDMH approved for IOBW and OLR poultry uses at 100 ppm.

FCN #453: DBDMH approved for general poultry water uses at 100 ppm.

FCN #775: DBDMH approved for use in ice in poultry establishments at 100 ppm.

FCN #792: DBDMH approved for beef carcass, parts and trim at 300 ppm.

Both Agencies have evaluated the efficacy of 1,3-dibromo-5, 5-dimethylhydantoin (DBDMH) very extensively. It is well known that DBDMH, when dissolved in water, combines with water to form hypobromous acid, which is the sole active ingredient of DBDMH. It is a very sparingly soluble, dry compound that dissolves very slowly and is difficult to use in a production environment without use of sophisticated and complex blending equipment to dissolve the FCS.

The hypobromous acid released during the use of DBDMH is exactly the same chemical compound as proposed in FCN Notification #944. There seems to be an impression that the two compounds are somehow different, which may be traced to the fact the source materials are different, but the fact remains that hypobromous acid is still hypobromous acid nonetheless.

With this in mind, we endeavored to perform a modest set of experiments conducted under more stringent environmental conditions than would be expected to occur under any set of circumstances. Namely, the meat and poultry products were sprayed with various pathogens and allowed to stand at room temperature and incubate over several hours. As one would expect, the pathogens would increase in numbers and enter further into the adipose or skin tissues. Then the samples were exposed to relatively short spray contact times at 60° F and enumerated.

Again, the objective was to establish relative comparisons rather than empirical or "white paper" results for the various antimicrobials under stressful conditions. In relation to hypobromous acid, we recognize the FSIS needs proof of efficacy suitability as a minimum baseline in order to assure a safe food supply. However, this applicant will again reiterate that this is not a new formulation or proprietary compound. The active ingredient, hypobromous acid, was evaluated by both Agencies in previous Food Contact Notifications listed above, and was determined to be safe and effective. This Applicant has requested that any and all efficacy or safety data that was submitted to the Agencies in support of DBDMH should and could be bridged with the data submitted in support of FCN #944, because the active ingredients are identical both factually and functionally.

Many of the above-mentioned similarity issues could also be made in relation to peroxyacetic acid. The Agencies undoubtedly are aware of the successful use of peroxyacetic acid in meat, poultry, seafood, fruit and vegetable facilities over the past decade or more. The original regulation was promulgated in 21 CFR 173.370, and closely followed by FCN #323 and #887, among others. The ceiling limits of peroxyacetic acid in almost all cases is about 220 ppm. We would suggest that the effects/efficacy of peroxyacetic acid should be viewed in total, including all other efficacy studies in the above-mentioned FCN and Directive 7120.1 files to give validation of the chemistry, and not one vendor's product vs. another's.

Further, we included bleach chlorine as one of the "yard-stick" comparative compounds in the poultry study. Although chlorine is not currently used preferentially on meat surfaces in the current production atmosphere, it is still broadly used in the poultry processing facilities at 50 ppm. This FCS was chosen as the most likely candidate for current and accepted use of a FCS antimicrobial compound as a "yard-stick". Note that bleach chlorine performed very modestly at best under the conditions of the submitted studies, and when viewed solely as a stand-alone antimicrobial compound, probably would be considered an unacceptable antimicrobial FCS by most standards, based on the study data results. We would again make the suggestion that this is further proof of the severity and difficult conditions this Applicant used in it's choices for a study protocol, and validates our position that we were looking for broad relative efficacy comparisons, rather than empirical efficacy numbers.

Comments on Efficacy Data (Intended Technical Effect)

Efficacy Data for Meat (Attachment #7 of the FCN)

Attachment #7 of the FCN was conducted on two separate days to rule out variables that can interfere with the outcome of the study. Because the two experiment days resulted in very little difference in inoculum concentrations, spray pressures, and microbiological results, it was decided that the data from the two experiment days, as well as the duplicate and triplicate testing of the meats be consolidated and the average results would be reported in that study. The average of all beef control results, the average of all pork control results, the average of all PAA, DBDMH and HB2-hypobromous acid-treated beef and pork results reported in the original submission can be seen in Tables 1 and 6. Because The Department of Health and Human Services requested additional data demonstrating statistical differences or variability within test groups, an expansion of the microbial results demonstrated in Tables 1 and 6 can be seen in Tables 2 – 5 and Tables 7 – 10, which is a collaboration of raw data that was consolidated and statistically evaluated to overcome efficacy deficiencies noted regarding Attachment 7.

In Experiment 1 of Appendix 7, immediately after each piece of meat was treated in the spray cabinet for 30 seconds, a water sample from the bottom of the drum was subjected to microbiological analysis. The average log 10 CFU/ml remaining, log 10 CFU/ml reduction and percent reduction from the wash water obtained at the bottom of the drum can be seen in Table 1. Tables 2 and 3 contain the actual log 10 CFU/ml remaining and log 10 CFU/ml reduction of wash water sampled from the bottom of the drum after each individual meat piece has been treated. It can be seen in Tables 2 and 3, that the standard deviation is less than 0.27 for each replicate when comparing the log 10 CFU/ml remaining and the log 10 CFU/ml reduction.

TABLE 1: Wash Water Microbiological Results From Bottom of Drum (Taken from Appendix 7 of the Original Submission) [CFU/ml]

Description	Log 10 CFU/ml *(remaining)*	Log 10 CFU/ml reduction	% reduction
Control Beef	5.01 avg.	N/A	N/A
plates	4.92; 5.19		
PAA Beef	0.24 avg.	4.77	99.998
plates	0.00; 0.47		
DBDMH Beef	0.48 avg.	4.53	99.997
plates	0.47; 0.85		
HB2 Beef	0.15 avg.	4.86	99.999
plates	0.00; .030		

Description	Log 10 CFU/ml *(remaining)*	Log 10 CFU/ml reduction	% reduction
Control Pork	5.27 avg.	N/A	N/A
plates	5.17; 5.36		
PAA Pork	0.00 avg.	>5.18	>99.999
plates	0.00; 0.00		
DBDMH Pork	0.99 avg.	4.19	99.994
plates	0.85; 1.38		
HB2	0.39 avg.	4.79	99.998
plates	0.30; 0.47		

TABLE 2: Wash Water Results From Bottom of Drum Beef Study Only: Revised to Include Statistical Analysis and All Replicates

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	
9/14/2009	Control Beef 1	10 psi @ 30 sec	4.92	
9/14/2009	Control Beef 2	10 psi @ 30 sec	5.19	
9/14/2009	Control Beef 1	70 psi @ 30 sec	4.85	
9/14/2009	Control Beef 2	70 psi @ 30 sec	5.07	
		Avg:	5.01	
		St. Deviation:	0.1524	

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	PAA Beef 1	10 psi @ 30 sec	0.00	4.77
9/14/2009	PAA Beef 2	10 psi @ 30 sec	0.47	
9/14/2009	PAA Beef 1	70 psi @ 30 sec	0.47	4.77
9/14/2009	PAA Beef 2	70 psi @ 30 sec	0.00	
		Avg:	0.24	4.77
		St. Deviation:	0.2714	0.0000

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	DBDMH Beef 1	10 psi @ 30 sec	0.85	4.35
9/14/2009	DBDMH Beef 2	10 psi @ 30 sec	0.47	
9/14/2009	DBDMH Beef 1	70 psi @ 30 sec	0.30	4.71
9/14/2009	DBDMH Beef 2	70 psi @ 30 sec	0.30	
		Avg:	0.48	4.53
		St. Deviation:	0.2594	0.2546

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	HB2 Beef 1	10 psi @ 30 sec	0.00	>5.06
9/14/2009	HB2 Beef 2	10 psi @ 30 sec	0.00	
9/14/2009	HB2 Beef 1	70 psi @ 30 sec	0.00	4.71
9/14/2009	HB2 Beef 2	70 psi @ 30 sec	0.30	
		*Avg:	0.08	4.86
		St. Deviation:	0.1500	0.2475

**Upon further calculations, it was determined that there was a calculation error and the average true log 10 CFU/ml remaining is actually 0.08 and not 0.15 as stated in the previous report.*

TABLE 3: Wash Water Results From Bottom of Drum Pork Study Only: Revised to Include Statistical Analysis and All Replicates

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	
9/14/2009	Control Pork 1	10 psi @ 30 sec	5.17	
9/14/2009	Control Pork 2	10 psi @ 30 sec	5.36	
9/14/2009	Control Pork 1	70 psi @ 30 sec	5.13	
9/14/2009	Control Pork 2	70 psi @ 30 sec	5.05	
		Avg:	5.18	
		St. Deviation:	0.1315	
Date of Exp	**Description**	**Experimental Conditions**	**Log 10 CFU/ml remaining**	**Log 10 CFU/ml reduction**
9/14/2009	PAA Pork 1	10 psi @ 30 sec	0.00	>5.18
9/14/2009	PAA Pork 2	10 psi @ 30 sec	0.00	
9/14/2009	PAA Pork 1	70 psi @ 30 sec	0.00	>5.18
9/14/2009	PAA Pork 2	70 psi @ 30 sec	0.00	
		Avg:	0.00	>5.18
		St. Deviation:	0.0000	0.0000
Date of Exp	**Description**	**Experimental Conditions**	**Log 10 CFU/ml remaining**	**Log 10 CFU/ml reduction**
9/14/2009	DBDMH Pork 1	10 psi @ 30 sec	0.85	4.06
9/14/2009	DBDMH Pork 2	10 psi @ 30 sec	1.38	
9/14/2009	DBDMH Pork 1	70 psi @ 30 sec	0.85	4.33
9/14/2009	DBDMH Pork 2	70 psi @ 30 sec	0.85	
		Avg:	0.98	4.19
		St. Deviation:	0.2650	0.1909
Date of Exp	**Description**	**Experimental Conditions**	**Log 10 CFU/ml remaining**	**Log 10 CFU/ml reduction**
9/14/2009	HB2 Pork 1	10 psi @ 30 sec	0.30	4.79
9/14/2009	HB2 Pork 2	10 psi @ 30 sec	0.47	
9/14/2009	HB2 Pork 1	70 psi @ 30 sec	0.47	4.79
9/14/2009	HB2 Pork 2	70 psi @ 30 sec	0.30	
		Avg:	0.39	4.79
		St. Deviation:	0.0981	0.0000

Tables 4 and 5 provide data that compare the microbiological results of the wash water tested from the bottom of the drum after chemical treatment at low pressure (10 psi) and high pressure (70 psi). The tables show that there is little difference between the use of high pressure and low pressure of chemically treating meat pieces.

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TABLE 4: Wash Water From Bottom of Drum Beef Study Only: Revised to Include Statistical Analysis Including Percent Difference Between Low (10 psi) and High Pressure (70 psi) Spray

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
Control	10 psi @ 30 sec	5.06 (4.92 + 5.19
Control	70 psi @ 30 sec	4.96 (5.07 + 4.85)
	St. Deviation:	0.0707
	% Difference:	1.98

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
PAA	10 psi @ 30 sec	0.24 (0.00 + 0.47)
PAA	70 psi @ 30 sec	0.24 (0.47 + 0.00)
	St. Deviation:	0.0000
	% Difference:	0.00

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
DBDMH	10 psi @ 30 sec	0.66 (0.85 + 0.47)
DBDMH	70 psi @ 30 sec	0.30 (0.30 + 0.30)
	St. Deviation:	0.2546
	% Difference:	54.5

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
HB2	10 psi @ 30 sec	0.15 (0.00 + 0.30)
HB2	70 psi @ 30 sec	0.15 (0.30 + 0.00)
	St. Deviation:	0.0000
	% Difference:	0.00

TABLE 5: Wash Water From Bottom of Drum Pork Study Only: Revised to Include Statistical Analysis Including Percent Difference Between Low (10 psi) and High Pressure (70 psi) Spray

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
Control	10 psi @ 30 sec	5.06 (4.92 + 5.19)
Control	70 psi @ 30 sec	5.09 (5.13 + 5.05)
	St. Deviation:	0.1273
	% Difference:	3.42

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
PAA	10 psi @ 30 sec	0.00 (0.00 + 0.00)
PAA	70 psi @ 30 sec	0.00 (0.00 + 0.00)
	St. Deviation:	0.0000
	% Difference:	0.00

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
DBDMH	10 psi @ 30 sec	1.12 (0.85 + 1.38)
DBDMH	70 psi @ 30 sec	0.85 (0.85 + 0.85)
	St. Deviation:	0.1909
	% Difference:	24.11

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
HB2	10 psi @ 30 sec	0.39 (0.30 + 0.47)
HB2	70 psi @ 30 sec	0.39 (0.47 + 0.30)
	St. Deviation:	0.0000
	% Difference:	0.00

After each piece of meat was sprayed with the test solution and taken back to the lab in sterile bags, 200g of city water was added to the bag and subsequently tumbled for one minute to dislodge any viable E. coli 0157:H7 bacteria. Table 6 was reported in Appendix #7 of the original submission and contains the average number of bacteria left on the meat after being sprayed for 30 seconds with either city water (control), PAA, DBDMH and HB2 solutions. Tables 7 and 8 contain the actual log 10 CFU/ml remaining and log 10 CFU/ml reduction of E. coli 0157:H7 bacteria left on each piece of beef and each piece of pork after treatment, respectively. It can be seen in Tables 7 and 8, that the standard deviation is less than 0.23 for each replicate when comparing the log 10 CFU/ml remaining and the log 10 CFU/ml reductions. This insignificant difference between replicates was the reasoning behind consolidating the data and subsequently making easier for the reader to follow.

TABLE 6: SPRAY Challenge Enumeration Results of Microbiological Analysis Summary: (Taken from Appendix #7 of the Original Submission)

Description	Log 10 CFU/ml (remaining)	Log 10 CFU/ml reduction	% reduction
Control Beef	6.15	N/A	N/A
PAA Beef	5.23	0.92	87.98
DBDMH Beef	5.56	0.59	74.30
HB2 Beef	5.45	0.70	80.03

Description	Log 10 CFU/ml (remaining)	Log 10 CFU/ml reduction	% reduction
Control Pork	6.43	N/A	N/A
PAA Pork	4.82	1.61	97.55
DBDMH Beef	5.71	0.72	80.95
HB2 Beef	5.25	1.18	93.39

TABLE 7: SPRAY Challenge Enumeration Results of Microbiological Analysis Beef Only (Revised to Include Statistical Analysis and All Replicates)

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/14/2009	Control Beef 1	10 psi @ 30 sec	6.16
9/14/2009	Control Beef 2	10 psi @ 30 sec	6.33
9/14/2009	Control Beef 1	70 psi @ 30 sec	5.94
9/14/2009	Control Beef 2	70 psi @ 30 sec	6.31
9/21/2009	Control Beef 1	40 psi @ 30 sec	6.22
9/21/2009	Control Beef 2	40 psi @ 30 sec	5.75
9/21/2009	Control Beef 3	40 psi @ 30 sec	6.28
		Avg:	6.14
		St. Deviation:	0.2174

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	PAA Beef 1	10 psi @ 30 sec	5.29	0.81
9/14/2009	PAA Beef 2	10 psi @ 30 sec	5.38	
9/14/2009	PAA Beef 1	70 psi @ 30 sec	5.36	0.89
9/14/2009	PAA Beef 2	70 psi @ 30 sec	5.14	
9/21/2009	PAA Beef 1	40 psi @ 30 sec	5.32	1.03
9/21/2009	PAA Beef 2	40 psi @ 30 sec	4.85	
9/21/2009	PAA Beef 3	40 psi @ 30 sec	5.17	
		Avg:	5.22	0.91
		St. Deviation:	0.1852	0.1118

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	DBDMH Beef 1	10 psi @ 30 sec	5.56	0.53
9/14/2009	DBDMH Beef 2	10 psi @ 30 sec	5.66	
9/14/2009	DBDMH Beef 1	70 psi @ 30 sec	5.56	0.64
9/14/2009	DBDMH Beef 2	70 psi @ 30 sec	5.44	
*9/21/2009	DBDMH Beef 1	40 psi @ 30 sec	...	N/A
*9/21/2009	DBDMH Beef 2	40 psi @ 30 sec	...	
*9/21/2009	DBDMH Beef 3	40 psi @ 30 sec	...	
		Avg:	5.56	0.59
		St. Deviation:	0.0900	0.0778

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	HB2 Beef 1	10 psi @ 30 sec	5.25	0.77
9/14/2009	HB2 Beef 2	10 psi @ 30 sec	5.50	
9/14/2009	HB2 Beef 1	70 psi @ 30 sec	5.25	0.83
9/14/2009	HB2 Beef 2	70 psi @ 30 sec	5.38	
9/21/2009	HB2 Beef 1	40 psi @ 30 sec	5.66	0.53
9/21/2009	HB2 Beef 2	40 psi @ 30 sec	5.54	
9/21/2009	HB2 Beef 3	40 psi @ 30 sec	5.62	
		Avg:	5.46	0.71
		St. Deviation:	0.1674	0.1540

** Note: The DBDMH active ingredient degraded by an unknown source of contamination when diluted and therefore that portion of this experiment, conducted 9/21/09, was not included as part of the results.*

TABLE 8: SPRAY Challenge Enumeration Results of Microbiological Analysis Pork Only (Revised to Include Statistical Analysis and All Replicates)

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/14/2009	Control Pork 1	10 psi @ 30 sec	6.79
9/14/2009	Control Pork 2	10 psi @ 30 sec	6.52
9/14/2009	Control Pork 1	70 psi @ 30 sec	6.52
9/14/2009	Control Pork 2	70 psi @ 30 sec	6.40
9/21/2009	Control Pork 1	40 psi @ 30 sec	6.16
9/21/2009	Control Pork 2	40 psi @ 30 sec	6.29
9/21/2009	Control Pork 3	40 psi @ 30 sec	6.31
		Avg:	6.43
		St. Deviation:	0.2056

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	PAA Pork 1	10 psi @ 30 sec	5.00	1.40
9/14/2009	PAA Pork 2	10 psi @ 30 sec	5.07	
9/14/2009	PAA Pork 1	70 psi @ 30 sec	4.83	1.67
9/14/2009	PAA Pork 2	70 psi @ 30 sec	4.70	
9/21/2009	PAA Pork 1	40 psi @ 30 sec	4.92	1.74
9/21/2009	PAA Pork 2	40 psi @ 30 sec	4.56	
9/21/2009	PAA Pork 3	40 psi @ 30 sec	4.59	
		Avg:	4.81	1.60
		St. Deviation:	0.1997	0.1815

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	DBDMH Pork 1	10 psi @ 30 sec	5.91	0.62
9/14/2009	DBDMH Pork 2	10 psi @ 30 sec	5.71	
9/14/2009	DBDMH Pork 1	70 psi @ 30 sec	5.69	0.81
9/14/2009	DBDMH Pork 2	70 psi @ 30 sec	5.56	
9/21/2009	DBDMH Pork 1	40 psi @ 30 sec	5.30	0.72
9/21/2009	DBDMH Pork 2	40 psi @ 30 sec	5.83	
9/21/2009	DBDMH Pork 3	40 psi @ 30 sec	6.00	
		Avg:	5.71	0.71
		St. Deviation:	0.2343	0.1308

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/14/2009	HB2 Pork 1	10 psi @ 30 sec	5.30	1.08
9/14/2009	HB2 Pork 2	10 psi @ 30 sec	5.41	
9/14/2009	HB2 Pork 1	70 psi @ 30 sec	5.19	1.19
9/14/2009	HB2 Pork 2	70 psi @ 30 sec	5.30	
9/21/2009	HB2 Pork 1	40 psi @ 30 sec	5.25	1.27
9/21/2009	HB2 Pork 2	40 psi @ 30 sec	5.19	
9/21/2009	HB2 Pork 3	40 psi @ 30 sec	5.05	
		Avg:	5.24	1.18
		St. Deviation:	0.1135	0.0962

Because there was no significant difference in the microbiological results between low pressure (10 psi) and high pressure (70 psi), the air pressure was set at 40 psi throughout Experiment 2 of Appendix #7 using triplicate meat and pork samples, rather than duplicates. Like Tables 4 and 5 on the previous pages, which compare the microbiological results with the use of low pressure and high pressure on the drum wash water only, Tables 9 and 10 demonstrate the percent difference and standard deviation between microbiological results that were actually left on the meat after treating at low pressure (10 psi) and high pressure (70 psi). There was a standard deviation of less than 0.1414 and a percent difference of less than 5.36% when comparing all samples and low and high pressure

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TABLE 9: SPRAY Challenge Enumeration Results of Microbiological Analysis Beef Study Only: Revised to Include Statistical Analysis Including Percent Difference Between Low (10 psi) and High Pressure (70 psi) Spray. Plate results reported in Tables #7 and #8 above.

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
Control	10 psi @ 30 sec	6.25
Control	70 psi @ 30 sec	6.13
	St. Deviation:	0.0849
	% Difference:	1.92

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
PAA	10 psi @ 30 sec	5.34
PAA	70 psi @ 30 sec	5.25
	St. Deviation:	0.0636
	% Difference:	1.69

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
DBDMH	10 psi @ 30 sec	5.61
DBDMH	70 psi @ 30 sec	5.50
	St. Deviation:	0.0778
	% Difference:	1.96

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
HB2	10 psi @ 30 sec	5.38
HB2	70 psi @ 30 sec	5.32
	St. Deviation:	0.0424
	% Difference:	1.12

TABLE 10: SPRAY Challenge Enumeration Results of Microbiological Analysis Pork Study Only: Revised to Include Statistical Analysis Including Percent Difference between Low (10 psi) and High Pressure (70 psi) Spray

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
Control	10 psi @ 30 sec	6.66
Control	70 psi @ 30 sec	6.46
	St. Deviation:	0.1414
	% Difference:	3.00

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
PAA	10 psi @ 30 sec	5.04
PAA	70 psi @ 30 sec	4.77
	St. Deviation:	0.1909
	% Difference:	5.36

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
DBDMH	10 psi @ 30 sec	5.81
DBDMH	70 psi @ 30 sec	5.63
	St. Deviation:	0.1273
	% Difference:	3.10

Description	Experimental Conditions	Log 10 CFU/ml remaining (avg.)
HB2	10 psi @ 30 sec	5.36
HB2	70 psi @ 30 sec	5.25
	St. Deviation:	0.0778
	% Difference:	2.05

Efficacy Data for Poultry (Attachment #8 of the FCN)

Attachment #8 of the FCN was conducted in duplicate using two different methods to determine the efficacy of four different chemistries against Salmonella typhimurium inoculated onto the surfaces of chicken halves. Because the duplicate measurements of the microbiological results showed little difference between replicates, it was decided that the data from duplicate testing of the chicken be consolidated and the average results would be reported in that study. The average of both chicken half control results and the averages of both PAA, DBDMH, NaOCl and HB2- hypobromous acid-treated poultry results reported in the original submission can be seen in Tables 11 and 13. Because The Department of Health and Human Services requested additional data demonstrating statistical differences or variability within test groups, an expansion of the microbial results demonstrated in Tables 11 and 13 can be seen in Tables 12 and 14, which is a collaboration of raw data that was consolidated and statistically evaluated to overcome efficacy deficiencies noted regarding Attachment #8.

Although there were only two replicates for each experimental condition, the data in Tables 12 and 14 which include the average and standard deviation of the log 10 CFU/ml remaining and log 10 CFU/ml reduction comparing each replicate was collaborated. Table 12 contains data from each replicate and each test substance when the chicken was treated by spray method at 40 psi. The standard deviation was less than 0.18 for the log 10 CFU/ml remaining and log 10 CFU/ml reduction for all samples. Table 14 shows that the standard deviation of the log 10 CFU/ml remaining and log 10 CFU/ml reduction was less than 0.14 for all test substances when the chicken was submerged for 40 minutes in chilled water.

TABLE 11: Microbiological Results from Chicken Treated by Spray Method (40 psi) Summary: (Taken From Attachment #8 of the Original Submission)

Description	Log 10 CFU/ml (remaining)	Log 10 CFU/ml reduction	% reduction
Control Chicken	6.15	N/A	N/A
PAA Chicken	5.30	0.85	85.87
DBDMH Chicken	5.85	0.30	49.88
NaOCl Chicken	5.77	0.38	58.31
HB2 Chicken	5.81	0.34	54.29

TABLE 12: Microbiological Results from Chicken Treated by Spray Method (40 psi): (Revised to Include Statistical Analysis and Both Replicates)

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	Control Chicken Half 1	40 psi @ 30 sec	6.28
9/22/2009	Control Chicken Half 2	40 psi @ 30 sec	6.02
		Avg:	6.15
		St. Deviation:	0.1838

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	PAA Chicken Half 1	40 psi @ 30 sec	5.33
9/22/2009	PAA Chicken Half 2	40 psi @ 30 sec	5.27
		Avg:	5.30
		St. Deviation:	0.0424

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	DBDMH Chicken Half 1	40 psi @ 30 sec	5.93
9/22/2009	DBDMH Chicken Half 2	40 psi @ 30 sec	5.77
		Avg:	5.85
		St. Deviation:	0.1131

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	NaOCl Chicken Half 1	40 psi @ 30 sec	5.78
9/22/2009	NaOCl Chicken Half 2	40 psi @ 30 sec	5.76
		Avg:	5.77
		St. Deviation:	0.0141

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	HB2 Chicken Half 1	40 psi @ 30 sec	5.85
9/22/2009	HB2 Chicken Half 2	40 psi @ 30 sec	5.76
		Avg:	5.81
		St. Deviation:	0.0636

TABLE 12: Microbiological Results from Chicken Treated by Spray Method (40 psi): (Revised to Include Statistical Analysis and Both Replicates)

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	Control Chicken Half 1	40 psi @ 30 sec	6.28
9/22/2009	Control Chicken Half 2	40 psi @ 30 sec	6.02
		Avg:	6.15
		St. Deviation:	0.1838

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	PAA Chicken Half 1	40 psi @ 30 sec	5.33
9/22/2009	PAA Chicken Half 2	40 psi @ 30 sec	5.27
		Avg:	5.30
		St. Deviation:	0.0424

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	DBDMH Chicken Half 1	40 psi @ 30 sec	5.93
9/22/2009	DBDMH Chicken Half 2	40 psi @ 30 sec	5.77
		Avg:	5.85
		St. Deviation:	0.1131

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	NaOCl Chicken Half 1	40 psi @ 30 sec	5.78
9/22/2009	NaOCl Chicken Half 2	40 psi @ 30 sec	5.76
		Avg:	5.77
		St. Deviation:	0.0141

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	HB2 Chicken Half 1	40 psi @ 30 sec	5.85
9/22/2009	HB2 Chicken Half 2	40 psi @ 30 sec	5.76
		Avg:	5.81
		St. Deviation:	0.0636

TABLE 13: Microbiological Results from Chicken Treated by Submersion Method (40 psi) Summary: (Taken From Attachment #8 of the Original Submission)

Description	Log 10 CFU/ml (remaining)	Log 10 CFU/ml reduction	% reduction
Control Chicken	6.54	N/A	N/A
PAA Chicken	5.50	1.04	90.88
DBDMH Chicken	6.25	0.29	48.71
NaOCl Chicken	6.08	0.46	65.33
HB2 Chicken	6.04	0.50	68.38

TABLE 14: Microbiological Results from Chicken Treated by Submersion Method (40 psi): (Revised to Include Statistical Analysis and Both Replicates)

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining
9/22/2009	Control Chicken Half 1	40 minute submersion	6.63
9/22/2009	Control Chicken Half 2	40 minute submersion	6.44
		Avg:	6.54
		St. Deviation:	0.1344

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/22/2009	PAA Chicken Half 1	40 minute submersion	5.48	1.06
9/22/2009	PAA Chicken Half 2	40 minute submersion	5.51	1.03
		Avg:	5.50	1.05
		St. Deviation:	0.0212	0.0212

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/22/2009	DBDMH Chicken Half 1	40 minute submersion	6.14	0.40
9/22/2009	DBDMH Chicken Half 2	40 minute submersion	6.35	0.19
		Avg:	6.25	0.30
		St. Deviation:	0.1485	0.1485

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/22/2009	NaOCl Chicken Half 1	40 minute submersion	6.16	0.38
9/22/2009	NaOCl Chicken Half 2	40 minute submersion	5.99	0.55
		Avg:	6.08	0.47
		St. Deviation:	0.1202	0.1202

Date of Exp	Description	Experimental Conditions	Log 10 CFU/ml remaining	Log 10 CFU/ml reduction
9/22/2009	HB2 Chicken Half 1	40 minute submersion	6.04	0.50
9/22/2009	HB2 Chicken Half 2	40 minute submersion	6.03	0.51
		Avg:	6.04	0.51
		St. Deviation:	0.0071	0.0071

Conclusions:

- The conditions used in these studies (Attachments #7 and #8) were severe compared to those expected in the "normal" process environment. Cold tap water or ice-chilled water was used throughout to treat the challenge food samples that were previously sprayed with >7 log10 CFU/ml amounts of E. coli or salmonella. The challenge samples were allowed to stand at room temperature for 2-3 hours before antimicrobial challenges were performed. The various food samples were processed and microbiological testing was performed in < 5 minutes after each challenge.
- Spraying is the almost exclusive methodology for treating meat with antimicrobial FCS treatments. Spraying of poultry with the FCS is also a substantial method of application for this food, along with submersion (i.e., such as a chiller). Using the spray methodology, Attachments #7 and #8 reported a substantial number of FCS challenge tests for each of the challenge food products. For meat challenges, 7 controls and 7 FCS challenges each were performed for PAA, DBDMH and hypobromous acid (HB2). The standard deviation was well within acceptable limits, with the control challenge resulting in the highest SD of 0.2174 for beef, and 0.2056 for pork, respectively. The SD for all other FCS challenges ranged from 0.0778 to a high of 0.1815.
- For chicken challenges, again the control had the highest standard deviation, at 0.1838. However, the highest SD for any of the FCS challenges was 0.1131, which occurred for DBDMH. For chilled submersion testing, the control group had a SD of 0.1344, which was exceeded only by that of DBDMH at 0.1485. The differences are statistically insignificant.
- For the chicken challenges, sodium hypochlorite and HB-2 performed almost identically, as one would expect, considering the chemistry of hypochlorous acid and hypobromous acid are similar in many respects.
- Since spraying methods of the FCS application are far and away the most common in meat, pork and chicken plants, the numerically significant number of challenges performed by this applicant should be sufficient evidence that the relative efficacy of the various FCS's has been established in this study, when viewed as a cumulative whole.
- We believe that the drum "Wash Water" analysis of the planktonic (unattached) pathogenic bacteria may not have been given the credit it is due in terms of evaluating over-all antimicrobial efficacy. This challenge was not as stressful and difficult as the sessile (attached) antimicrobial studies. But this analysis has the advantage of expressing the basic and functional efficacy characteristics of each of the FCS's. All the FCS's performed very well over short periods of time in the submitted studies and this data should be viewed cumulatively with the other challenges.
- The FCS's used in this study are very commonly accepted and reliable antimicrobial treatments in meat and poultry plants. Many of the FCS's have been used for many years with success in process facilities, and we believe we have shown the relative efficacy of hypobromous acid and PAA to be quite similar and effective in these scenarios.