



**ABC Research Corporation**

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(Originally American Bacteriological & Chemical Research Corporation)

April 19, 2001

Dr. Ken Byrd  
Pilgrim's Pride  
P.O. Box 93  
Pittsburg, TX 75686  
Tel: 903-855-4335  
Fax: 214-565-2305

Dear Ken,

Please find enclosed the project report for the study entitled "Evaluation of the Antimicrobial Efficacy of Mionix Spray Treatment for Reduction of Selected Microorganisms on Fresh Chicken Carcasses" as well as a statement of Project Quality Assurance and a copy of raw data records.

We at ABC Research appreciate this opportunity to do business with Pilgrim's Pride and Mionix. Please let us know if you have any questions or what further services we can provide.

Sincerely,

James E. (Ken) Kennedy, Jr., Ph.D.  
Director, Research Microbiology

cc: Mr. Michael Cunha - Mionix  
Dr. Wafa Bibari - ABC Research  
Dr. Bill Brown - ABC Research  
Ms. Patricia Baxter - ABC Research

enclosure

4/19/2001



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**PROJECT REPORT  
RESEARCH MICROBIOLOGY DEPARTMENT  
ABC RESEARCH CORPORATION**

DATE: April 19, 2001

PREPARED FOR: Pilgrim's Pride

CLIENT CONTACT: Dr. Ken Byrd

PROJECT: Evaluation of the Efficacy of Mionix Spray Treatment for Reduction of Selected Microorganisms on Fresh Chicken Carcasses.

OBJECTIVE: The objective of this study was to evaluate the antimicrobial efficacy of a Mionix spray and/or rinse treatment in the reduction of *Salmonella*, generic *E. coli* and Aerobic Plate Count on Fresh Chicken Carcasses.

EXPERIMENTAL APPROACH:

A. PRODUCT:

Fresh, warm eviscerated chicken carcasses (n=160 total) with crops removed were collected from a local commercial slaughter facility. Carcasses were collected immediately after inside and outside water rinsing and before any antimicrobial spray and chilling. Samples were selected for standard size and lack of obvious defects. They were individually bagged in plastic and held in insulated containers until inoculation and treatment. Collection, transport to the laboratory, and storage prior to treatment required up to four hours. Eighty carcasses were collected on one day for uninoculated studies and eighty the next day for inoculated studies.

B. TEST MICROORGANISMS:

Two strains of *Salmonella* were used in this study as listed below.

1. *Salmonella enteritidis* (ATCC 13076, nalidixic acid resistant)
2. *Salmonella typhimurium* (ATCC 14028, nalidixic acid resistant)

Two strains of generic *E. coli* were used in this study as listed below.

1. *E. coli* (ATCC 15597)
2. *E. coli* (ATCC 12435)

4/19/2001

### C. INOCULATION PROCEDURE:

*Salmonella* cultures were individually propagated in Brain Heart Infusion broth (BHI) plus 200 ppm nalidixic acid (BHI + Nal) and incubated at 35°C for 24 h before the experiment. *E. coli* cultures were individually propagated in Brain Heart Infusion broth (BHI) and incubated at 35°C for 24 h before the experiment. Cells were harvested by centrifugation at 10,000 x g for 10 min. and washed twice with Butterfield's Phosphate Buffer, pH 7.2 (BPB). *Salmonella* and *E. coli* cells from each culture were resuspended and pooled in 400 ml BPB to obtain a cell suspension of ca.  $1.0 \times 10^9$  CFU/ml for each microorganism. Sufficient volume (i.e., 400 ml) of this suspension was added to a reservoir tank of ca. 4 liters of 0.1% peptone buffer to yield a concentration of ca.  $1.0 \times 10^8$  colony forming units (CFU) of each bacterial strain per ml of cocktail.

Carcasses were inoculated by immersion in the cocktail for 30 seconds, hung on the shackles, and allowed to air dry at ambient temperature for 10-15 min. Carcasses were then treated according to protocol described below.

### D. MIONIX TREATMENT

Mionix Corporation provided "Mionix Formulation No. 4" for application in this study. The procedure for the spray treatment of 80 carcasses (inoculated or uninoculated) on a given day is summarized below.

1. Carcasses were hung by their hocks on shackles for spray/rinse treatment.
2. For the forty Mionix treated carcasses, the outside of each carcass was thoroughly sprayed from hocks to neck with the Mionix solution for 5 sec. on each side using two electric sprayers delivering a total of approximately 400 ml to the carcass.
3. Immediately after spraying the outside of the carcass, the visceral cavity was completely filled with the Mionix solution (ca. 400 ml) while pinching the neck cavity closed and then allowed to drain via the neck cavity.
4. The control group of 40 untreated carcasses received the same spray/rinse treatment as described in item 2 (above) but with deionized water instead of Mionix solution.
5. After spray/rinse treatment, the carcasses were allowed to drip for two minutes.
6. After dripping, twenty "pre-chill" carcasses were microbiologically analyzed.
7. After dripping, the other 20 carcasses were immersed in iced tap water for one hour to simulate commercial chilling but without any water disinfectant. The iced water was stirred every 10-15 min. to simulate plant conditions.
8. After chilling, the twenty "post-chill" carcasses were allowed to drip for two minutes before microbial analyses.

Inoculation/treatment/chilling combinations are summarized below.

1. Uninoculated, pre-chill, Mionix treated carcasses (n=20)
2. Uninoculated, pre-chill, water (control) treated carcasses (n=20)
3. Uninoculated, post-chill, Mionix treated carcasses (n=20)
4. Uninoculated, post-chill, water (control) treated carcasses (n=20)

5. Inoculated, pre-chill, Mionix treated carcasses (n=20)
6. Inoculated, pre-chill, water (control) treated carcasses (n=20)
7. Inoculated, post-chill, Mionix treated carcasses (n=20)
8. Inoculated, post-chill, water (control) treated carcasses (n=20)

#### E. SAMPLE ANALYSES:

Carcasses were microbiologically analyzed using a modification of the USDA rinse technique with 400ml of Difco Neutralizing Buffer. For inoculated carcasses, *Salmonella* was enumerated using XLD plates containing 200 ppm nalidixic acid and generic *E. coli* was enumerated with Petrifilm™. Uninoculated samples were enumerated for Aerobic Plate counts.

#### RESULTS:

Microbiological results for *Salmonella* inoculated carcasses are presented in Table 1. For pre-chill carcasses, *Salmonella* counts for control (water treated) samples ranged from 4.52 to 6.03 log<sub>10</sub> units per ml of sample rinse with a mean of 5.07 log<sub>10</sub> units per ml as compared to a mean and range of 3.32 log<sub>10</sub> units per ml and 2.34-4.46 log<sub>10</sub> units per ml, respectively, in the Mionix treated samples. A similar trend was noted for the post chill carcass samples although the differences between control and Mionix treated samples were not as great as with the pre-chill samples. Mean *Salmonella* counts for control and Mionix treated post-chill carcasses were 3.90 and 3.72 log<sub>10</sub> units per ml, respectively. Statistical analyses (i.e., Students *t* test) evidenced that the pre- and post-chill Mionix treated samples had significantly lower (P<0.01) *Salmonella* counts than corresponding control samples. It should also be noted that *Salmonella* counts were significantly lower on post-chill samples than on corresponding pre-chill samples for both control and Mionix treated carcasses.

Microbiological results for *E. coli* inoculated carcasses are presented in Table 2. For pre-chill carcasses, *E. coli* counts for control samples ranged from 6.00 to 8.18 log<sub>10</sub> units per ml with a mean of 7.03 log<sub>10</sub> units per ml. In contrast, Mionix treated samples had a mean and range of 4.48 log<sub>10</sub> units per ml and 4.20-5.30 log<sub>10</sub> units per ml, respectively. A similar trend was noted for the post chill carcass samples. Mean *E. coli* counts for control and Mionix treated post-chill carcasses were 5.76 and 4.45 log<sub>10</sub> units per ml, respectively. The pre- and post-chill Mionix treated samples had significantly lower (P < 0.01) *E. coli* counts than corresponding control samples. *E. coli* counts were also significantly lower on post-chill samples than on corresponding pre-chill samples for both control and Mionix treated carcasses.

Microbiological results for Aerobic Plate Counts (APC's) on uninoculated carcasses are presented in Table 3. For pre-chill carcasses, APC's for control samples ranged from 5.98 to 7.20 log<sub>10</sub> units per ml with a mean of 6.70 log<sub>10</sub> units per ml as compared to a mean and range of 6.17 log<sub>10</sub> units per ml and 5.53-6.65, log<sub>10</sub> units per ml, respectively, in the Mionix treated samples. This difference was significant (P<0.01). However, the Mionix treated, post-chill carcasses had slightly higher APC's than corresponding control samples. APC's were

also significantly lower on post-chill samples than on corresponding pre-chill samples for both control and Mionix treated carcasses.

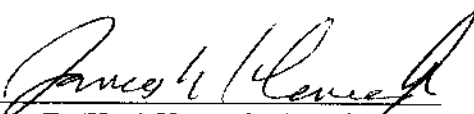
SUMMARY:

*Salmonella* and *E. coli* counts were significantly lower on Mionix treated carcasses than on control (water treated) carcasses before and after chilling with larger differences being noted in pre-chill samples. Aerobic Plate Counts of uninoculated carcasses were significantly lower on Mionix treated samples than on control samples for pre-chill but not post-chill carcasses. The chilling step resulted in a significant decrease in corresponding microbial counts on carcasses for all treatment groups.

DISCLAIMER:

Neither ABC Research Corporation, nor any of its employees, makes any claims concerning the results generated from this project. Acceptance of the data generated by any government agency is the sole responsibility of Mionix and Pilgrim's Pride.

PREPARED BY:



James E. (Ken) Kennedy, Jr., Ph.D.  
Director, Research Microbiology

**Table 1. *Salmonella* Counts on Fresh Chicken Carcasses Before and After Chilling With and Without Mionix Treatment.**

	Pre - Chill				Post - Chill			
	Control (water treated)		Mionix Treated		Control (water treated)		Mionix Treated	
	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml
Rep 1	77,000	4.89	220	2.34	12,300	4.09	4,100	3.61
Rep 2	192,000	5.28	3,010	3.48	10,400	4.02	5,800	3.76
Rep 3	33,000	4.52	1,470	3.17	12,200	4.09	4,400	3.64
Rep 4	30,000	4.48	1,080	3.03	10,700	4.03	8,700	3.94
Rep 5	272,000	5.43	2,030	3.31	7,800	3.89	7,400	3.87
Rep 6	40,000	4.60	2,100	3.32	7,100	3.85	2,700	3.43
Rep 7	58,000	4.76	2,680	3.43	8,400	3.92	8,200	3.91
Rep 8	1,060,000	6.03	1,640	3.21	18,600	4.27	4,000	3.60
Rep 9	500,000	5.70	2,100	3.32	7,800	3.89	5,400	3.73
Rep 10	740,000	5.87	3,060	3.49	6,400	3.81	8,900	3.95
Rep 11	104,000	5.02	3,820	3.58	3,400	3.53	6,200	3.79
Rep 12	170,000	5.23	2,480	3.39	5,000	3.70	4,600	3.66
Rep 13	870,000	5.94	1,040	3.02	7,100	3.85	9,500	3.98
Rep 14	520,000	5.72	3,100	3.49	7,300	3.86	4,400	3.64
Rep 15	85,000	4.93	620	2.79	5,800	3.76	2,600	3.41
Rep 16	48,000	4.68	5,960	3.78	8,600	3.93	7,500	3.88
Rep 17	42,000	4.62	28,800	4.46	8,400	3.92	3,500	3.54
Rep 18	33,200	4.52	850	2.93	4,200	3.62	8,200	3.91
Rep 19	40,000	4.60	2,600	3.41	12,400	4.09	4,500	3.65
Rep 20	45,000	4.65	2,660	3.42	9,040	3.96	3,000	3.48
<b>Log Mean</b>	--	<b>5.07</b>	--	<b>3.32</b>	--	<b>3.90</b>	--	<b>3.72</b>
<b>Std Dev</b>	--	0.53	--	0.42	--	0.17	--	0.18
<b>Geom. Mean</b>	--	117,000	--	2,090	--	7,900	--	5,250
<b>t value (log<sub>10</sub> values)</b>	11.61				3.32			
<b>Significance</b>	Control > Treated @ 0.01 level				Control > Treated @ 0.01 level			

**Table 2. *E. coli* Counts on Fresh Chicken Carcasses Before and After Chilling With and Without Mionix Treatment.**

	Pre - Chill				Post - Chill			
	Control (water treated)		Mionix Treated		Control (water treated)		Mionix Treated	
	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml
Rep 1	1,000,000	6.00	20,000	4.30	310,000	5.49	19,000	4.28
Rep 2	3,200,000	6.51	200,000	5.30	460,000	5.66	20,000	4.30
Rep 3	2,000,000	6.30	16,000	4.20	1,900,000	6.28	20,000	4.30
Rep 4	2,000,000	6.30	17,000	4.23	3,400,000	6.53	18,000	4.26
Rep 5	50,000,000	7.70	16,000	4.20	560,000	5.75	180,000	5.26
Rep 6	44,000,000	7.64	32,000	4.51	380,000	5.58	21,000	4.32
Rep 7	35,000,000	7.54	29,000	4.46	300,000	5.48	20,000	4.30
Rep 8	80,000,000	7.90	16,000	4.20	480,000	5.68	30,000	4.48
Rep 9	150,000,000	8.18	32,000	4.51	1,900,000	6.28	18,000	4.26
Rep 10	18,000,000	7.26	16,000	4.20	460,000	5.66	40,000	4.60
Rep 11	4,000,000	6.60	21,000	4.32	520,000	5.72	16,000	4.20
Rep 12	80,000,000	7.90	55,000	4.74	400,000	5.60	17,000	4.23
Rep 13	38,000,000	7.58	16,000	4.20	400,000	5.60	10,000	4.00
Rep 14	100,000,000	8.00	100,000	5.00	600,000	5.78	220,000	5.34
Rep 15	60,000,000	7.78	20,000	4.30	68,000	4.83	16,000	4.20
Rep 16	1,600,000	6.20	32,000	4.51	2,100,000	6.32	100,000	5.00
Rep 17	2,600,000	6.41	40,000	4.60	480,000	5.68	210,000	5.32
Rep 18	2,300,000	6.36	25,000	4.40	560,000	5.75	20,000	4.30
Rep 19	1,700,000	6.23	170,000	5.23	440,000	5.64	13,000	4.11
Rep 20	1,600,000	6.20	16,000	4.20	800,000	5.90	8,000	3.90
<b>Log Mean</b>		<b>7.03</b>		<b>4.48</b>		<b>5.76</b>		<b>4.45</b>
<b>Std Dev</b>		<b>0.77</b>		<b>0.34</b>		<b>0.37</b>		<b>0.43</b>
<b>Geom. Mean</b>		<b>10,700,000</b>		<b>30,200</b>		<b>575,000</b>		<b>28,200</b>
<b>t value (log<sub>10</sub> values)</b>	<b>13.57</b>				<b>10.33</b>			
<b>Significance</b>	<b>Control &gt; Treated @ 0.01 level</b>				<b>Control &gt; Treated @ 0.01 level</b>			

**Table 3. Aerobic Plate Counts on Fresh Chicken Carcasses Before and After Chilling With and Without Mionix Treatment.**

	Pre - Chill				Post - Chill			
	Control (water treated)		Mionix Treated		Control (water treated)		Mionix Treated	
	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml	cfu/ml	log <sub>10</sub> /ml
Rep 1	6,400,000	6.81	2,340,000	6.37	1,220,000	6.09	260,000	5.41
Rep 2	4,000,000	6.60	2,160,000	6.33	350,000	5.54	1,020,000	6.01
Rep 3	4,960,000	6.70	2,010,000	6.30	250,000	5.40	320,000	5.51
Rep 4	960,000	5.98	1,540,000	6.19	62,000	4.79	160,000	5.20
Rep 5	4,160,000	6.62	1,040,000	6.02	70,000	4.85	92,000	4.96
Rep 6	2,240,000	6.35	340,000	5.53	31,000	4.49	520,000	5.72
Rep 7	11,160,000	7.05	1,140,000	6.06	280,000	5.45	740,000	5.87
Rep 8	16,000,000	7.20	2,720,000	6.43	350,000	5.54	1,280,000	6.11
Rep 9	2,660,000	6.42	1,160,000	6.06	1,540,000	6.19	640,000	5.81
Rep 10	3,200,000	6.51	2,580,000	6.41	250,000	5.40	600,000	5.78
Rep 11	7,520,000	6.88	720,000	5.86	80,000	4.90	760,000	5.88
Rep 12	6,560,000	6.82	1,600,000	6.20	120,000	5.08	310,000	5.49
Rep 13	12,640,000	7.10	2,240,000	6.35	60,000	4.78	16,000,000	7.20
Rep 14	8,160,000	6.91	1,920,000	6.28	620,000	5.79	288,000	5.46
Rep 15	1,600,000	6.20	1,120,000	6.05	76,000	4.88	320,000	5.51
Rep 16	2,060,000	6.31	4,480,000	6.65	30,000	4.48	400,000	5.60
Rep 17	12,000,000	7.08	1,440,000	6.16	19,000	4.28	14,080,000	7.15
Rep 18	2,900,000	6.46	2,240,000	6.35	39,000	4.59	336,000	5.53
Rep 19	10,400,000	7.02	340,000	5.53	68,000	4.83	270,000	5.43
Rep 20	10,080,000	7.00	1,920,000	6.28	22,000	4.34	40,000	4.60
<b>Log Mean</b>		<b>6.70</b>		<b>6.17</b>		<b>5.08</b>		<b>5.71</b>
<b>Std Dev</b>		<b>0.34</b>		<b>0.28</b>		<b>0.56</b>		<b>0.61</b>
<b>Geom. Mean</b>		<b>5,010,000</b>		<b>1,480,000</b>		<b>120,000</b>		<b>513,000</b>
<b>t value (log<sub>10</sub> values)</b>	<b>5.36</b>				<b>- 3.40</b>			
<b>Significance</b>	<b>Control &gt; Treated @ 0.01 level</b>				<b>Treated &gt; Control @ 0.01 level</b>			





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### STATEMENT OF PROJECT QUALITY ASSURANCE

ABC Research Corporation is a full service, ISO accredited laboratory (see attached). The Research Microbiology Department of ABC Research Corporation operates under a Good Laboratory Practice policy. Raw data entry, transcriptions, calculations, statistical analyses and final data presentations are reviewed and verified for accuracy by the Department Director and/or Manager prior to issuance of a final project report. The subject review and verification have been conducted on the data for the project entitled "Evaluation of the Efficacy of Mionix Spray Treatment for Reduction of Selected Microorganisms on Fresh Chicken Carcasses" as indicated below.

PROJECT DATA VERIFIED BY:

James E. (Ken) Kennedy, Jr., Ph.D.  
Director, Research Microbiology

PROJECT DATA VERIFIED BY:

Dcstry Taylor  
Manager, Research Microbiology