# IEH Laboratories & Consulting Group

Final Report

Efficacy of Hypobromous Acid as a Hide Intervention

Performed July 18-20, 2011

Submitted to
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Prepared by:
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# Laboratories & Consulting Group

July 29, 2011


Submitted to
Mike Harvey
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Re: Final Report for Third Party Efficacy of Hypobromous Acid as a Hide Intervention


Mike,


IEH Laboratories & Consulting Group (IEH) is pleased to submit this Final Report for a Third Party Study for efficacy of hypobromous acid as a hide intervention.


If you have any questions or require further information, please do not hesitate to contact me directly.

Sincerely,


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**Objective** – The Enviro Tech wanted to determine the antimicrobial efficacy of the application of hypobromous acid (HB2) on cattle hides.

**Experimental Design** – Enviro Tech wanted to evaluate the effect of 220 ppm and 500 ppm of HB2 in reducing indicators organisms (Total Plate Counts – TPC, Total Coliforms Count, TCC and E. coli Count, ECC.) well as *E. coli* O157:H7 and *Salmonella*. To determine the effect of HB2, 99 hide samples were removed before and after the application of HB2 for each of 220 and 500 ppm concentrations.

**Materials and Methods** –

**HB2 Application** - At a beef processing plant, whole pulled hides were draped over barrels to simulate hide-on carcasses for evaluation of the effect of HB2 on TPC, TCC, ECC, *E. coli* O157:H7 and *Salmonella*. One side of the hide was used for 220 ppm concentration effect determination and the other side of the hide was used for the 500 ppm concentration effect determination. Two samples per hide side were obtained per concentration. HB2 was delivered with a pump with the pressure of 45 PSI at the source (6 gallons per min). The application time was 15 seconds and the dwell time was 2 min. Throughout the sampling time measurements were made to ensure that proper concentration of HB2 was delivered. The pH of both solutions were measured periodically during the trial. Both solutions were maintained between pH 7.2-7.4. No pH adjustments were necessary.

**Sampling -** All hide samples were collected using Speci-Sponge Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) from about 500-cm$^2$ areas.

**Bacterial counts -** Aerobic plate counts (APC), *E. coli* counts, and total coliform counts were determined using Petrifilm count plates (3M Microbiology, St. Paul, Minn.). *E. coli* O157:H7 and *Salmonella* enumeration and prevalence were determined using the protocol described by Arthur et al. 2007 - (T.A. Arthur, J.M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S.D. Shackelford, T. L. Wheeler and M. Koohmaraie. 2007. Effects of a Minimal Hide Wash Cabinet on the Levels and Prevalence of Escherichia coli O157:H7 and Salmonella on the Hides of Beef Cattle at Slaughter. J. Food Protection Vol. 70, No. 5, Pages 1076-1079).

**Data Analysis** – For indicators organisms (APC, TCC and ECC), all values were converted to log10 count/sample. Log transformed data were analyzed using 2-independent sample student "t" test (Microsoft Excel Statistic). This yielded mean log values, mean log differences, standard deviations, and p-values associated with the test statistic. In short, the p-value provides a measure of probability that a difference between two experimental groups occurred by chance. Thus, the lower the p-value, the more likely differences observed between population means were due to the independent variable(s) [e.g. treatment(s) evaluated].

**Results** – The results for the effect of HB2 on indicator organisms are reported in Table 1 and 2 for 220 ppm and 500 ppm, respectively. Treating cattle hides with 220 ppm of HB2 for 15 sec and 2 min dwell time reduced APC, TCC and ECC counts by 2.21, 2.21 and 2.13 logs,

respectively.  Treating cattle hides with 500 ppm of HB2 for 15 sec and 2 min dwell time reduced APC, TCC and ECC counts by 3.31, 2.21 and 3.83 logs, respectively.

The results for the effects of HB2 on pathogens of concerns to public health and thus of major interest to beef processing industry are reported in table 3 and 4 for the two concentrations of HB2.  Application of 220 ppm HB2 reduced *E. coli* O157:H7 and *Salmonella* prevalence by 60% and 75%, respectively.

In addition to prevalence, we also quantified the levels of *E. coli* O157:H7 and *Salmonella*.  The sensitivity of the method (lower detection limit) is 40 CFU/100 $cm^2$ of hide.  That is if each of these pathogens are present at concentration higher than 40 CFU/100 $cm^2$ of hide, they will be shown as enumerable.

The population of *E. coli* O157:H7 was below the 40 CFU/100 $cm^2$ for all hides used in this project.  Thus treatment effect cannot be determined.  However, this was not the case for *Salmonella*.  For 220 ppm HB2 control 19 hides, were enumerable and treating these hides with 220 ppm of HB2 for 15 seconds and 2 min of dwell time decreased the *Salmonella* enumerable number of hide samples to 4.  Thus treating hides with 220 ppm HB2 reduce the number of hides with enumerable *Salmonella* by 80%.

For 500 ppm HB2 control, 10 hides were enumerable for *Salmonella* and treating these hides with 500 ppm of HB2 for 15 seconds and 2 min of dwell time decreased the *Salmonella* enumerable number of hide samples to 7 hides.  Thus treating hides with 500 ppm HB2 reduce the number of hides with enumerable *Salmonella* by 30%.

**Conclusions** – These results clearly indicate that HB2 can be an effective intervention for hide decontamination at the beef processing plant.  Both concentrations of HB2 reduced the indicators as well as pathogens.  The lower concentration of HB2 was less effective against indicator organisms but equal in effect or more effective against pathogens than the high concentration of HB2.  Without further investigations, an explanation for the differences in effectives of the two concentrations of HB2 used in this study is not apparent at this time.  Though possible, it is implausible for the lower concentration to be more effective against pathogens tested than the high concentration used.  The effect of 500 ppm HB2 on indicator organisms is certainly impressive (3.31, 3.72 and 3.83 logs reduction for APC, TCC and ECC, respectively).  Over the years this investigator has been involved in evaluating the effect of many compounds for their suitability as a hide intervention.  Some of these compounds include sodium hydroxide, tri-sodium phosphate, chlorinate, ozonated and electrolyzed water (see the attached publications).  The results presented here indicate that HB2 are equal in effect or more effective that the above compounds.  These results indicate that HB2 (hypobromous acid) would could be a valuable tool for reducing microbiological contamination in a hide washer system immediately prior to processing. It appears that HB2 is at least equal to or more effective than the above listed compounds that are used for hide-on washers.

Table 1. Mean ± SD of APC, TCC and ECC (Log CFU/sponge) from carcass samples taken before and after the application of 220 ppm HB2.

|  | APC Log CFU/sponge | TCC Log CFU/sponge | ECC Log CFU/sponge |
|---|---|---|---|
| Before (n=99) | $9.08 \pm 0.44^a$ | $7.18 \pm 0.85^a$ | $7.05 \pm 0.84^a$ |
| After (n=99) | $6.87 \pm 1.36^b$ | $4.97 \pm 1.52^b$ | $4.82 \pm 1.50^b$ |
| Reduction | 2.21 | 2.21 | 2.23 |

[a,b] Means, within column, lacking common superscript letters, differ ($P \le 0.05$).

Table 2. Mean ± SD of APC, TCC and ECC (Log CFU/sponge) from carcass samples taken before and after the application of 500 ppm HB2.

|  | APC Log CFU/sponge | TCC Log CFU/sponge | ECC Log CFU/sponge |
|---|---|---|---|
| Before (n=99) | $9.29 \pm 0.58^a$ | $7.15 \pm 0.93^a$ | $7.01 \pm 0.91^a$ |
| After (n=99) | $5.98 \pm 0.90^b$ | $3.43 \pm 1.27^b$ | $3.18 \pm 1.30^b$ |
| Reduction | 3.31 | 3.72 | 3.83 |

[a,b] Means, within column, lacking common superscript letters, differ ($P \le 0.05$).

Table 4. *E. coli* O157:H7 and *Salmonella* prevalence on cattle hides before and after the application 220 ppm HB2.

|  | *E. coli* O157:H7 Prevalence (%) | *E. coli* O157 Enumerable | *Salmonella* Prevalence (%) | *Salmonella* Enumerable |
|---|---|---|---|---|
| Before (n=99) | $25.3^a$ | 0 | $28.3^a$ | $19^a$ |
| After (n=99) | $10.1^b$ | 0 | $7.1^b$ | $4^b$ |
| Reduction | 60 | - | 74.9 | 80 |

Table 5. *E. coli* O157:H7 and *Salmonella* prevalence on cattle hides before and after the application 500 ppm HB2.

|  | *E. coli* O157:H7 Prevalence (%) | *E. coli* O157 Enumerable | *Salmonella* Prevalence (%) | *Salmonella* Enumerable |
|---|---|---|---|---|
| Before (n=99) | $21.2^a$ | 0 | $33.3^a$ | $10^a$ |
| After (n=99) | $10.1^b$ | 0 | $8.1^b$ | $7^b$ |
| Reduction | 52.3 | - | 75.6 | 30 |