Safety Assessment of Hypobromous Acid (220 ppm as Br_2) Used as a Beef Carcass Wash

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Chlorine, in its gaseous form, or as hypochlorous acid (HOCl), has long been used as disinfectant because of its microbiocidal activity and ready availability. In particular it is widely used to treat drinking water, in swimming pools, and as a carcass wash in meat and poultry processing plants. While the health benefits of these uses in eliminating microbial pathogens is clear, these benefits must be balanced against the potential hazards from the disinfection byproducts (DBPs) that arise when chlorine reacts with naturally occurring organic material in the water. These include trihalomethanes that form as a result of reaction of HOCl with substances containing a secondary carbonyl group. The most common of these trihalomethanes produced in drinking water disinfection is chloroform ($CHCl_3$). Mixed trihalomethanes can also form if the source water contains trace amounts of bromide ion. Bromine itself (or hypobromous acid) can also react in the same way to form tribromomethane (bromoform). Trihalomethanes, and other DBPs are of concern because some are potentially carcinogenic (http://www.epa.gov/enviro/html/icr/dbp_health.html).

Because of this, it is important to determine whether the use of hypobromous acid as a carcass wash might result in hazardous levels of DBPs being formed on the meat. To examine this, EnviroTech has performed a series of investigations to determine to what extent trihalomethanes, particularly bromoform, are formed during use of hypobromous acid (220 ppm as Br_2) as a beef carcass wash.

In the initial investigation, as the carcasses emerged from the carcass wash process employing 220 ppm HOBr (as available Br_2), the drippings from 5 carcasses from the carcass wash line were combined and shaken and then used to fill special environmental vials of 40 ml volume. This was performed 4 times each day for 5 days for a total of 20 samples overall, representing a total of 100 carcasses sampled over the 5 day period. Samples of plant process water and carcass wash water that had not contacted a carcass were also collected to establish the baseline or background level of THMs already present in the water (the control). The carcass wash dripping samples were kept refrigerated until all 20 had been collected. They were then shipped overnight on ice to Aquatic Research Inc. in Seattle, WA to determine the total THM present in each sample. Aquatic Research, Inc. is a specialist in the field of THM analysis. In total, 28 samples (see Table 1) were analyzed by Aquatic Research Inc.

Table 1 Summary of Samples Analyzed in First Investigation	
Number of Samples Analyzed	**Sample Description**
5	Method Blanks (prepared samples with no THMs)
2	Control Carcass Wash Solution (HOBr solution (220 ppm as Br_2) that had not contacted a carcass.)
2	Control Process Water Solution (processing plant source water used for preparing the carcass wash)
20	Carcass Wash Drippings (collected from carcasses after treatment with HOBr [220 ppm as Br_2])

The samples were analyzed using EPA Method 8260. THMs were determined by measurement of purgeable organic compounds using a capillary column on a Gas Chromatography-Mass Spectrometry (GC-MS) instrument. The carcass wash dripping were diluted 1:1 before injection onto the column and were adjusted by the 50% dilution factor.

The reported total THMs was derived from the sum of 4 compounds: bromodichloromethane, bromoform, dibromochloromethane, and chloroform. The detection limit of the Chromatography/Mass Spectrometry instrument used to obtain the results for the individual compounds was 2.0 µg/L.

Table 2 reports the average concentrations of bromodichloromethane, bromoform, dibromochloromethane, and chloroform for each sample set; method blank, control process water, control carcass wash, and carcass wash drippings.

Table 2 Summary of Results of First Investigation				
Sample Description	**Trihalomethanes (Average concentration - µg/L)**			
	Chloroform	**Bromo-dichloromethane**	**Dibromo-chloromethane**	**Bromoform**
Method Blanks	< detection limit*	< detection limit	< detection limit	< detection limit
Control Process Water	< detection limit	< detection limit	< detection limit	< detection limit
Control Carcass Wash	1.25	< detection limit	< detection limit	171
Carcass Wash Drippings	< detection limit	< detection limit	0.56	93.29
* Detection limit = 2.0 µg/L				

As shown in Table 2, no trihalomethanes were detected in the method blank and control process water samples, demonstrating the validity of the assay procedure. In the control carcass wash samples (i.e. the 220 ppm as Br2 solution of HOBr that had not contacted an animal carcass), one sample had no detectable chloroform while the other contained chloroform just above the detection limit at 2.5 µg/L. The concentration of bromoform in these samples was not detectable in one and 342 µg/L in the other.

The average of the 20 carcass wash samples was 0.56 µg/L dibromochloromethane and 93.29 µg/L bromoform.

Because of the inconsistent results with the control carcass wash samples, a second round of testing was performed in which five separate samples of control carcass wash were collected (i.e., HOBr solution containing 220 ppm Br2) as prepared for use as a carcass wash at the same beef processing facility. The results are summarized in Table 3.

Table 3 Summary of Results of Second Investigation

Sample Description	Trihalomethanes (Average concentration ± SD-- µg/L)			
	Chloroform	**Bromo-dichloromethane**	**Dibromo-chloromethane**	**Bromoform**
Method Blank	< detection limit*	< detection limit	< detection limit	< detection limit
Control Carcass Wash	6.2 ± 0.29	5.26 ± 0.52	6.64 ± 0.70	304.2 ±139.0

* Detection limit = 2.0 µg/L

The high concentrations of bromoform in the control carcass wash suggested the possibility of some concern, but also raised the question of whether the THMs were being formed at the time the carcass wash solution was prepared (and could, therefore contact, and potentially contaminate the carcasses), or whether it was formed slowly, in the dark, in the sealed sample vials during shipping to the analytical lab.

To investigate this question, and to assess whether THMs potentially formed in the carcass wash were preferentially absorbed by the outer fat layer of the carcass, a third round of sampling was conducted.

To address the first question, two classes of samples of hypobromous acid were collected. All samples were collected from the processing floor over a period of several hours.

1. Without carcass contact: The hypobromous acid-water mixture was sampled without contact with the animal carcasses. These samples were collected at 5 separate times, and each sample was divided into two 40 ml vials (total of 10 vials). One of each pair of vials was sterile only, and the other vial contained 200 mg of ascorbic acid to neutralize free hypobromous acid without interfering with the test analysis equipment (and without affecting any THMs that may already have been formed).
2. With carcass contact: These samples were collected at 5 separate times and contained the hypobromous acid wash solution after being used to wash the beef carcass, and each sample also contained ascorbic acid to neutralize the remaining hypobromous acid and prevent artefactual formation of THMs during sample shipment.

If there was a mass balance in THMs, i.e. the ascorbic acid-neutralized control carcass wash THMs equals the THMs in the carcass wash drippings, then THMs would NOT have been preferentially absorbed from the aqueous phase into the outer fat layer of the carcass. If there was NOT a mass balance in THMs i.e. the ascorbic acid-neutralized control carcass wash THMs > THMs in carcass wash drippings, then THMs may have been preferentially absorbed from the aqueous phase into the outer fat layer of the carcass.

To further address the question of possible absorption of THMs by fat, in another set of experiments performed at the laboratory, pieces of beef adipose tissue (22 cm2) were added to two of the 40 ml vials from the samples obtained without carcass contact and without ascorbic acid). This simulated the volume of water-fat surface area that would occur when 20 gallons of carcass wash contacts the exposed 45 ft2 surface of each ½ carcass in the spray cabinet. After the fat was added, the vials were recapped and gently agitated for two minutes to ensure that all of the solution in the vial had contacted the adipose tissue. After two minutes, the adipose tissue was removed from the vial and the solutions were immediately re-analyzed for THMs. Any substantial depletion of THMs in the sample exposed to the adipose tissue was recorded. The difference in THM concentration between the samples with and without contact with adipose tissue permits estimation of the THM concentration in the outermost fat layer of tissue, if it were absorbed.

The same analytical procedures were used as in the earlier rounds of sampling. The results of the first group of samples (control carcass wash that did not contact carcasses, and collected with or without ascorbic acid) are summarized in Table 4.

Table 4: Group 1 Average Concentrations of THMs Analyzed				
Sample Description	**Trihalomethanes (average µg/L ± SD)**			
	Chloroform	**Bromo-dichloromethane**	**Dibromo-chloromethane**	**Bromoform**
Method Blank	< detection limit	< detection limit	< detection limit	< detection limit
Control Carcass Wash Solutions A (ascorbic acid neutralized)	< detection limit	2.40 ± 0.34	2.68 ± 0.74	< detection limit
Control Carcass Wash Solutions B (NOT ascorbic acid neutralized)	< detection limit	3.88 ± 0.23	5.72 ± 0.045	201.8 ± 58.3
Carcass Wash Drippings (ascorbic acid neutralized)	< detection limit	< detection limit	< detection limit	18.8 ± 11.1

As in the second round of sampling, control carcass wash solution (220 ppm as Br_2 solution of HOBr that had not contacted an animal carcass) that was collected without ascorbic acid showed elevated levels of brominated THMs, particularly bromoform (average, 202 µg/L). In contrast, when ascorbic acid was present in the sample vial, the concentration of THMs was close to or below the detection limit. These results indicate that the elevated levels of THMs found in the control carcass wash in the absence of ascorbic acid represent the formation of THMs in the sample vials during shipment, and do not reflect concentrations to which the meat carcasses would have been exposed.

Also shown in Table 4 are the results of the analysis of carcass wash samples collected into vials containing ascorbic acid after contact with a carcass. In these samples, chloroform, bromodichlormethane and dibromochloromethane were not detectable. Bromoform was also not detectable in one of the five samples, but was detected at concentrations ranging between 15 and 30 µg/L in the other four samples.

To evaluate whether any of the THMs found in the carcass wash drippings would tend to adsorb to adipose tissue, the second phase of this study in which two samples of control carcass wash solution collected without ascorbic acid, and therefore containing elevated levels of THMs by the time it reached the analytical lab, were exposed to portions of adipose tissue in an amount designed to mimic the proportions of carcass wash volume per carcass. The concentrations of

bromoform in these samples before and after exposure to the adipose tissue are shown in Table 5.

Table 5 Comparison of Bromoform Concentration (µg/L) in Carcass Wash Solution Before and After Contact with Adipose Tissue			
	Before	**After**	**Difference**
Sample 1	82.3	83.8	+ 1.5
Sample 2	113	95.8	– 15.2

For Sample 1, there was virtually no difference in the bromoform level between before and after exposure of 40 ml of solution to a 22 cm2 piece of beef adipose tissue that was agitated for 2 minutes. However, for Sample 2 there was a 15.2 µg/L decrease in bromoform concentration after the same test with another solution with a similar level of bromoform. When Aquatic Research (the lab performing the testing) was asked whether this difference was significant, the response was negative. It was pointed out that the measurement is reproducible to within ± 5 % per replicate. Further, the testing with adipose tissue was an experiment, and not a Standard Method of Analysis. This introduced variables such as existence of a headspace in the vial and interferences from the presence of adipose tissue.

While there does not appear to be evidence of strong absorption of bromoform to adipose tissue, the results shown in Table 4 for the carcass wash drippings indicates that there is some exposure of sprayed carcasses to bromoform. To provide a cautious assessment of the potential health risk that might result from this exposure, we consider the situation where a sprayed carcass retains some of the wash solution, and that solution contains bromoform at 18.8 µg/L, as seen in the carcass wash drippings shown in Table 4.

An average whole beef carcass after processing weighs approximately 800 lbs, but only half carcasses (400 lbs, 181.6 kg) are sprayed with 220 ppm HOBr (as Br_2) in the spray cabinet. Further, industry assumptions are that the carcass retains 0.3 % of the weight of liquid applied to it during this initial process. Thus it can be calculated the carcass retains 1.2 lbs, or 0.547 L, of carcass wash fluid.

The concentration of bromoform per unit weight of meat (µg/kg) was calculated by multiplying the average concentration of bromoform in the carcass wash residual solution (18.8 µg/L) by the volume of fluid retained on a half carcass (0.547 L) and dividing it by the weight of a half carcass (181.6 kg). The calculated concentration of bromoform per unit weight of meat is thus:

Bromoform Concentration in meat=18.8 (μg/L) × 0.547(L) ÷ 181.6 (kg) = 0.057 μg/kg.

For a 70 kg person consuming four ounces (0.113 kg) of such beef per day, their daily dose of bromoform would be 0.057 x 0.113/70 = 9.2×10^{-5} μg/kg/day, or 9.2×10^{-8} mg/kg/day. This calculation includes the very conservative assumption that the bromoform would remain with the meat, and not dissipate during further processing, storage, or cooking.

To assess whether such a dose of bromoform presents a health risk to a consumer, we need to consider two aspects of the toxicology of bromoform. The US Environmental Protection Agency (http://www.epa.gov/iris/subst/0214.htm) has evaluated the toxicity of bromoform and derived an estimate of the daily dose that is free of any non-cancer toxic effects. This is called the Reference Dose (RfD) and is defined by EPA as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." Because bromoform also causes intestinal tumors in rats receiving chronic exposure to high doses, EPA has also derived for bromoform an estimate of the upper limit on the "oral slope factor" that defines its carcinogenic potency.

For the non-cancer effects of bromoform, the most sensitive endpoint (i.e., the effect occurring at the lowest dose) following prolonged, repeated exposure was histopathological changes in the liver in rats. Based on this, EPA derived an RfD of 2×10^{-2} mg/kg/day. This dose is hundreds of thousands of times higher than the dose of bromoform (9.2×10^{-8} mg/kg/day) calculated above that might result from use of hypobromous acid as a carcass wash. Hence, no non-cancer health effects would be expected from this trace level of bromoform.

USEPA has also derived an oral cancer slope factor for bromoform of 7.9×10^{-3} $(mg/kg/day)^{-1}$. Based on this value, the lifetime cancer risk for someone eating four ounces of this beef every day for a lifetime would be:

9.2×10^{-8} mg/kg/day x 7.9×10^{-3} $(mg/kg/day)^{-1}$ = 7.3×10^{-10}, i.e., less than one in a billion.

This is far below the level of risk that is of any public health concern. Overall, there is no reason to believe that the trace levels of bromoform, or other bromination byproducts, that might occur in meat as a result of the use of a hypobromous acid carcass was (220 ppm as Br_2) could present any health risk to consumers of the meat.