

Disinfection of Poultry Chiller Water with Chlorine Dioxide: Consumption and Byproduct Formation

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Chlorine dioxide is effective in controlling the natural flora occurring in poultry chiller water (PCW). However, it is not approved for use as a disinfectant because of the lack of information pertinent to its human health risks. In PCW, most added chlorine dioxide was rapidly reduced to chlorite and chlorate. The formation of chlorite was dependent on both chlorine dioxide dose and treatment time. The chlorine dioxide demand by PCW, the consumption at a given treatment time, was found to be independent of treatment dose. When used at the demand level, half of the added chlorine dioxide was reduced to a mixture of chlorite (90%) and chlorate (10%). On the basis of the acceptable daily intake of chlorite, 0.075 mg/kg, disinfecting PCW at the level of chlorine dioxide demand, 0.30 mM (20 mg/L), is not only effective but also has a low health risk from the standpoint of chlorite and chlorate contamination.

Keywords: Chlorine dioxide; poultry chiller water; consumption; demand; chlorite; chlorate

INTRODUCTION

Statistically, each person in the United States consumes 44 kg (96 lb) of poultry meat annually. Poultry accounts for about 36% of meat consumption, second only to beef in the American diet. The wholesomeness of poultry products has a profound impact on public safety and health. The U.S. poultry industry produces 20 billion pounds of chicken and 6 billion pounds of turkey each year. Almost all poultry products are produced in "ready-to-cook" forms from automated plants of multimillion bird capacity. In these plants birds are slaughtered, defeathered, eviscerated, rinsed, chilled, and packed. Chilling carcasses rapidly to below 40 °F is crucial for minimizing microbial growth and preserving carcass quality. It is accomplished by immersing rinsed carcasses in icy water in one, two, or three long tanks, the chillers. Many processors use chlorine to control microbial populations in poultry chiller water (PCW). Presently, chlorine and its hydration products, hypochlorous acid and hypochlorite, are the only disinfectants permitted by the regulatory agencies for use in PCW (U.S. Department of Agriculture, 1993).

Poultry chiller water is known for its high content of organic matter (Tsai et al., 1992). Chlorination of PCW results in the formation of trihalomethanes, primarily chloroform (Robinson et al., 1981; Tsai unpublished data), and other mutagenic compounds that have yet to be identified (Masri, 1986; Schade et al., 1990). Although the health impact of these potentially deleterious compounds has not been established, providing alternative methods for disinfecting PCW is highly desirable.

Chlorine dioxide is a potential substitute for chlorine. Baron et al. (1973) reported adding chlorine dioxide to chiller water or to holding water is more efficient than using in-plant chlorination to reduce bacteria in water samples and from turkey skin surfaces. Lillard (1979)

reported chlorine dioxide is 7 times more effective than chlorine in controlling aerobic bacteria in PCW. In a separate study, Lillard (1980) reported that *Salmonella* incidence in chicken was reduced to zero by treating PCW with 5 mg/L chlorine dioxide. Thiessen et al. (1984), on the other hand, reported 1.39 mg/L was sufficient to achieve the same results. *Salmonella* control on turkey carcasses by treatment of PCW with chlorine dioxide surpassed that achieved by in-plant chlorination treatment (Villarreal et al., 1990).

Chlorine dioxide, like chlorine, is an oxidant, but its redox potential in aqueous solution, 1.15 V ($\text{ClO}_2 + e = \text{ClO}_2^-$), is less than that of hypochlorous acid, 1.49 V ($\text{HClO} + \text{H}^+ + 2e = \text{Cl}^- + \text{H}_2\text{O}$) (White, 1986). Therefore, it is likely to be less reactive and produce fewer byproducts than chlorine in PCW. There has been little information on trihalomethane formation in chlorine dioxide treated water. Robinson et al. (1981) found trace amounts of chloroform in chlorine dioxide water containing chicken carcasses. They attributed the chloroform to the chlorine that contaminated the chlorine dioxide solution. Ghanbari et al. (1982) reported that the chlorine atom in chlorine dioxide incorporated much more slowly with unsaturated lipids than chlorine itself in aqueous solution. Tan et al. (1987) reported that chlorine dioxide is relatively inert compared to chlorine in reacting with individual amino acids. However, both chlorine and chlorine dioxide react very quickly with peptides and proteins. It was impossible to differentiate the reaction rates.

The primary reduction byproducts of chlorine dioxide during disinfection are chlorite and chlorate (Masselein, 1979). In 1982, the National Research Council (1982) suggested both 24-h and 7-day SNARLs (Suggested No-Adverse-Response Levels) for chlorite in drinking water to be 0.125 mg/L. The same SNARLs were recommended for chlorate. In 1993 the U.S. Environmental Protection Agency proposed new Disinfectants/Disinfection Byproducts Rules to be implemented in 1998. The maximum contaminant level (MCL) for chlorite in drinking water will be set at 1.0 mg/L (U.S. Environmental Protection Agency, 1993).

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Table 1. Approximate Analysis (Percent) of Poultry Chiller Water^a

sample	screened ^b				membrane filtered ^c			
	total solids	fat	ash	total nitrogen	total solids	fat	ash	total nitrogen
PCW-177	0.105	0.056	0.045	0.0034	0.100	0.039	0.044	0.0032
PCW-178	0.118	0.043	0.048	0.0016	0.101	0.014	0.067	0.0018
PCW-179	0.087	0.021	0.044	0.0014	0.079	0.019	0.049	0.0012
mean	0.103	0.040	0.046	0.0021	0.093	0.024	0.053	0.0021
SD	0.014	0.016	0.002	0.0010	0.011	0.012	0.011	0.0009
CV, %	14	40	4	45	12	50	21	46
		unscreened ^d						
mean	0.350	0.196	0.115	0.0148				
SD	0.141	0.096	0.067	0.0185				
CV, %	40	49	58	125				

^a Average of duplicates. ^b Screened with No. 35 sieve. ^c Filtered through 0.45 μm and then 0.2 μm Duroapore membranes. ^d Previously reported by Tsai et al. (1992).

Chlorate was not considered in the same proposal. The acceptable daily intake of chlorite in food calculated as part of a recent food additive petition was determined to be 0.075 mg/kg (Rio Linda Chemical Co., Inc., 1994).

Because chlorine dioxide is potentially a better disinfectant than chlorine in food-processing water, its consumption in PCW along with chlorite and chlorate formation was systematically investigated and is reported here. Additional studies on chlorine dioxide disinfection, such as its efficiency, mutagenicity, and oxidative effects, will be covered in forthcoming papers.

MATERIALS AND METHODS

Poultry Chiller Water. The water sample was obtained from the prechiller of a local commercial broiler processor which was described previously (Tsai et al., 1987). PCW samples were collected in Teflon bottles from the side-trough after the chiller had operated for 5 (or more) h from the beginning of the shift. Samples, packed in ice, were transferred to the authors' laboratory, immediately screened with a No. 35 sieve (500 μm opening, U.S. Standard Testing Sieve, W. S. Tyler, Inc., Mentor, OH), stored at 2–4 °C, and used within 7 days. For proximate analysis, a portion of the screened PCW was filtered with a membrane of 0.45 μm pore opening (Durapore, Millipore, Bedford, MA) using a Minitan Acrylic Ultrafiltration System (Millipore) and then filtered through a 0.2 μm membrane filter for storage.

Proximate Analysis. PCW screened with a No. 35 sieve and with 0.45 and 0.2 μm membranes was analyzed for the content of solids, total nitrogen, ash, and lipids. Methods of the Association of Official Analytical Chemists (AOAC, 1980) were used to determine total solids (Section 33.041), total nitrogen (Section 2.057), and ash (Section 14.006). Lipid content was determined from the total dry solids (Section 7.056).

Chlorine Dioxide Preparation. Chlorine dioxide solution, free from hypochlorite, was prepared by slowly adding 23 mL of hydrochloric acid (3 M) to 80 mL of 7.5% sodium chlorite solution (Masschelein, 1979, p 123) in a 150 mL round-bottom flask. The chlorine dioxide generated was stripped by N_2 and passed through three glass traps (40 mL in volume) connected in series into 125 mL of organic-free water in a glass cylinder kept in crushed ice. Traps 1 and 3 were empty. Trap 2 contained 20 mL of 10% sodium chlorite and 1% sodium hydroxide. The concentration of chlorine dioxide in the cylinder reached 1.0–1.3% in 60 min. This stock solution was stable for several months when kept in a sealed amber bottle under refrigeration.

Residual Chlorine Dioxide Determination. PCW at room temperature, 20–22 °C, was mixed with a calculated amount of chlorine dioxide stock solution to give the desired concentration of chlorine dioxide treatment. At various treatment times, an aliquot of the mixture was drawn and diluted to 200 mL with organic-free water for the determination of residual chlorine dioxide using an amperometric titration

method (Aieta et al., 1984). Titrant, phenylarsine oxide solution (0.00564 N, Shape Products Co., Oakland, CA), was added automatically with a Metrohm Titroprocessor (Brinkmann Instrument Co., Westbury, NY) at 0.05 mL increments. At pH 7, each milliliter of phenylarsine oxide used is equivalent to 0.028 mM or 1.9 mg/L chlorine dioxide. The optimum range of response for the Metrohm Titroprocessor is between 0 and 0.056 mM (3.8 mg/L) chlorine dioxide. Titration accuracy is ± 0.0014 mM (0.095 mg/L) chlorine dioxide, or 5% at 0.028 mM. Chlorine dioxide stock solution was titrated at pH 7 and then at pH 2 before it was used. The ratio of titrant at pH 2 to that at pH 7 must be 4 ± 0.1 to ensure the chlorine dioxide solution was free from chlorine contamination (Aieta et al., 1984). Since chlorine is carefully excluded, measuring at pH 7 presumably reflects only chlorine dioxide. Blank titration indicates the absence of any detectable oxidant in PCW.

Removal of Residual Chlorine Dioxide in Aqueous Solution. A 25 mL sample of 0.63 mM (42.5 mg/L) chlorine dioxide was placed in a vial and nitrogen gently bubbled into it through a coarse sinter glass tip. Every 5 min thereafter, an aliquot (ca. 1 mL) was withdrawn and the chlorine dioxide was titrated amperometrically.

For the determination residual chlorite and chlorate in chlorine dioxide treated PCW, 25 mL of the treated PCW was purged in a similar manner for 30 min before they were determined with the method of high-performance liquid chromatography.

Chromatographic Determination of Chlorite and Chlorate in Chlorine Dioxide Treated PCW. To a series of PCW samples (20 mL each) in 25 mL volumetric flasks was added chlorine dioxide stock solution of known concentration, and the flask was brought to volume with organic-free water. At the prescribed time, a flask was purged with nitrogen for 30 min. After being filtered through an "On-Guard Ag" cartridge (Dionex Corp., Sunnyvale, CA), the PCW was analyzed with a high-performance liquid chromatography system (Dionex Ion Chromatography System 4000, Dionex Corp.) equipped with a Dionex AS9-SC column and a conductivity detector (Pfaff et al., 1989). The series were duplicated, and each sample of the duplicated runs was analyzed for chlorite and chlorate contents in triplicate. The means and 95% confidence intervals of the triplicates were plotted using Sigmaplot for Windows (Jandel Scientific, San Rafael, CA).

RESULTS AND DISCUSSION

Composition of Poultry Chiller Water. The approximate analysis of the PCW used in the current study is presented in Table 1. The same table also consists of the accumulated analysis of PCW from the same chiller reported previously (Tsai et al., 1992). The solid content reported here is recognized to be the highest throughout the chiller because they were collected from the side-trough of the chiller, where the solids content was the highest. It is also because the

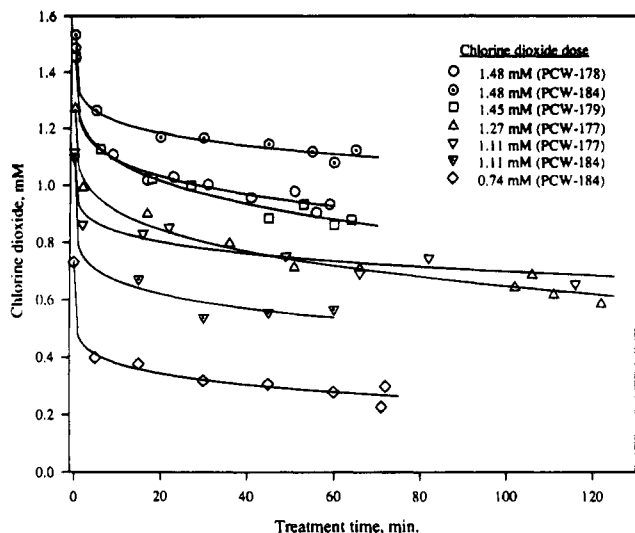


Figure 1. Chlorine dioxide consumption in poultry chiller water.

PCW samples were collected after the solid content had plateaued (5 to 6 h of operation). The PCW used in the current study was screened with a No. 35 sieve that excludes particles of size $>500 \mu\text{m}$, while the PCW used previously was not subject to such pre-treatment. Screening resulted in a more homogeneous mixture as indicated by visual observation and by lower standard deviations (Table 1). Compared to mean results for unscreened PCW's used in earlier studies, screening appeared to remove a significant amount of solids: about 70% total solids, 89% fat, 86% total nitrogen and 60% ash. Table 1 also shows the composition of clear filtrate obtained by membrane filtration ($0.2 \mu\text{m}$ pore size). Membrane filtration did not remove any more ash and total nitrogen from screened PCW, but did remove some fat, which mostly were globules of diameter ranging from 0.2 to $500 \mu\text{m}$. These results suggest that screened PCW differs from clear membrane filtrate only in fat content. The solids content of the three screened PCW's varied from 0.087 to 0.118%, but their ash contents were quite similar. The fat content of PCW-179 was about a half of PCW-177 and 178. The total nitrogen of PCW-177 is about twice that of PCW-178 and 179.

Residual Chlorine Dioxide. Figure 1 shows that the PCW samples were treated with four levels of chlorine dioxide: 1.48 mM (100 mg/L), 1.27 mM (86 mg/L), 1.11 mM (75 mg/L), and 0.74 mM (50 mg/L). The treatment with 1.48 mM chlorine dioxide was duplicated in PCW-178 and PCW-184. The treatment of PCW-179 with 1.45 mM chlorine dioxide was in reality another replicate because chlorine dioxide can only be determined with $\pm 5\%$ accuracy. Treatment with 1.11 mM chlorine dioxide was duplicated in PCW-177 and PCW-184. Treatments with 1.27 and 0.74 mM were performed singularly in PCW-177 and PCW-184, respectively.

Regardless of treatment concentration, chlorine dioxide rapidly decreased initially (Figure 1). The rates of decrease flatten after 20 min. Analyzed mathematically with a data fitting program, TableCurve 2D (Jandel Scientific), the relation of residual chlorine dioxide and treatment time was found to be best expressed with

$$Y = A + BT^C \quad (1)$$

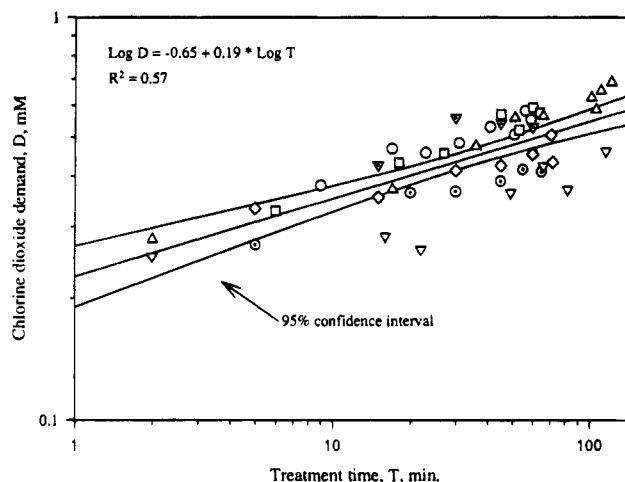


Figure 2. Chlorine dioxide demand of poultry chiller water.

Table 2. Best Regression Fit, $Y = A + BX^C$, of Residual Chlorine Dioxide in Poultry Chiller Water with Time

sample and treatment	A	-B	SE	C	SE	R ²
1.48 mM (PCW-184)	1.54	0.208	0.029	0.173	0.029	0.9878
1.48 mM (PCW-178)	1.49	0.257	0.036	0.193	0.032	0.9860
1.45 mM (PCW-179)	1.45	0.212	0.032	0.243	0.033	0.9883
1.27 mM (PCW-177)	1.27	0.207	0.035	0.238	0.031	0.9829
1.11 mM (PCW-177)	1.11	0.192	0.055	0.169	0.053	0.9386
1.11 mM (PCW-184)	1.10	0.315	0.132	0.142	0.099	0.9789
0.74 mM (PCW-184)	0.74	0.260	0.043	0.138	0.033	0.9804
av		0.236		0.185		
SD		0.044		0.042		

where Y represents residual chlorine dioxide (mM), T is treatment time (min), and A , B , and C are coefficients. The smooth lines in Figure 1 shows the regression curves of best fit. The coefficients are presented in Table 2. According to eq 1, coefficient A equals the concentration of chlorine dioxide when T equals zero, i.e., the dose or treatment concentration. Coefficients B and C turn out to be quite uniform in the range of chlorine dioxide tested (Table 2).

Most chlorine dioxide treatments were stopped at 120 min or sooner. Tests with 1.48 (PCW-178) and 1.27 mM (PCW-177) were extended to 1020 and 1230 min, respectively. Extending treatment time 10-fold had no apparent effect on the results of regression analysis. The equation derived from the data obtained in about 120 min of treatment may be used to predict concentration of residual chlorine dioxide in PCW for at least 5 times the treatment period.

Chlorine Dioxide Demand. Equation 1 may be rearranged as

$$A - Y = -BT^C \quad (2)$$

The difference between added and residual chlorine dioxide, $A - Y$, is referred to as chlorine dioxide demand. It is a function of time but independent of the added chlorine dioxide. Chlorine dioxide demand, D , may be expressed by the following linear equation:

$$\log D = \log(-B) + C(\log T) \quad (3)$$

Equation 3 can be used to predict demand or to calculate coefficients B and C , as in Figure 2, on the basis of the residual chlorine dioxide determined analytically. The chlorine dioxide demands of the current PCW at 3, 18, and 60 min were 0.28, 0.39, and 0.49 mM, respectively. Because these values were derived from the water collected from the location having the highest solid

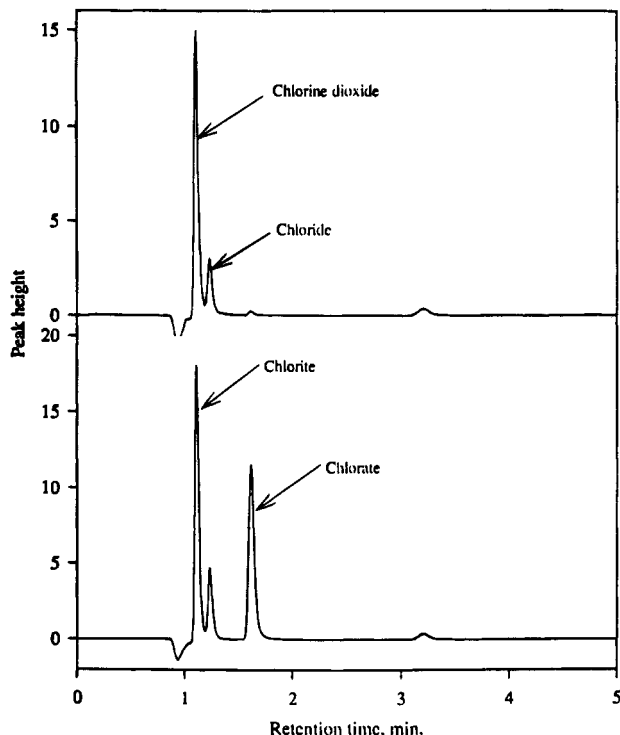


Figure 3. Separation of chlorine dioxide, chloride, chlorite, and chlorate by high-performance liquid chromatograph using Dionex Ion Chromatography System 4000 with Dionex AS9-SC column, conductivity detector, and isocratic elution, NaHCO_3 (0.2 mM)/ Na_2CO_3 (1.4 mM), at 1 mL/min.

content, they represent the maximum chlorine dioxide demands that the chiller water may reach at different treatment times.

Normally, disinfectant is fed into the chiller via makeup water or via a separate water stream near the makeup water. At the makeup water inlet, which is usually also the terminal end of chilling process for carcasses, the contact time between chlorine dioxide and PCW is short. The chlorine dioxide demand at this point is best represented by the value of short treatment time, for example, 3 min. The chlorine dioxide demand is expected to be less than 0.28 mM because the solid content of the water at this location is expected to be always lower than the PCW used in the study. At the opposite end of the chiller, where the water overflows and carcasses come in, the chlorine dioxide demand of the water may be best represented with treatment time that equals the residence time of water in chiller. If the residence time of chiller water is assumed to be the same as that of the carcasses it is displaced by, the demand of PCW at the overflow drain would be approximately 0.49 mM, because the residence time of carcasses is approximately 60 min in most commercial settings.

Chlorite and Chlorate Formation. In chromatographic determination of the byproducts of chlorine dioxide disinfection, residual chlorine dioxide interfered with chlorite quantification because both have the same retention time (Figure 3). Fortunately, chlorine dioxide is very volatile and can be readily purged out of aqueous solution with gentle bubbling of nitrogen. Figure 4 shows the concentration of residual chlorine dioxide in 25 mL of 42.5 mg/L chlorine dioxide solution gently bubbled with nitrogen. The results demonstrate that purging for 30 min removed 98% of the chlorine dioxide, and for a purge of 60 min, 99% was removed. A 30 min purge was chosen to remove the residual chlorine

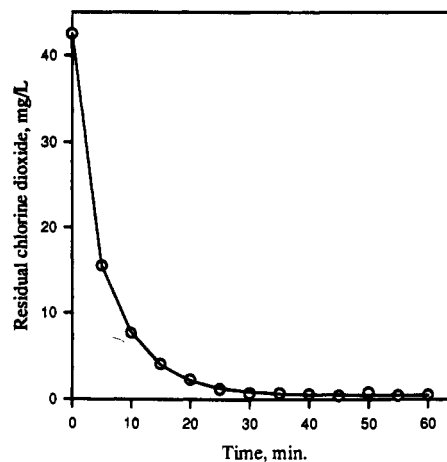


Figure 4. Residual chlorine dioxide in aqueous solution during nitrogen purging.

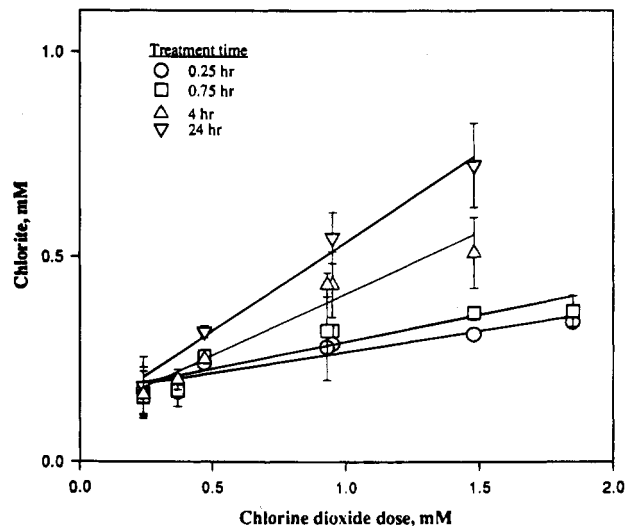


Figure 5. Chlorite in chlorine dioxide treated poultry chiller water. The solid lines represent the linear regression of the data (symbols). The error bars indicate 95% confidence intervals of the analysis in triplicate.

dioxide for the chromatographic determination of chlorite and chlorate.

Figure 5 shows that the chlorite content in chlorine dioxide treated PCW increased linearly with increasing dose of chlorine dioxide. Chlorite formation also increased significantly with increasing treatment time. However, the difference with respect to treatment time diminished when the concentration of chlorite dropped to 0.2 mM or below, i.e. when the PCW was treated with approximately 0.4 mM or less chlorine dioxide. The indifference of chlorite formation to treatment time at low concentration of chlorine dioxide treatment may be due to the low sensitivity of the analytical method, which is indicated by the overlapping 95% confidence intervals.

In chlorine dioxide treated PCW, the amount of chlorate formed was about 10% that of chlorite. The marginally formed chlorate did not appear to be affected significantly by treatment time but was related to the chlorine dioxide dose (Figure 6).

The percentage of chlorine dioxide detected as chlorite and chlorate is shown in Table 3. When the added chlorine dioxide was below the demand of PCW, e.g. 0.24 mM, about 70% of the added chlorine dioxide converted to chlorite and chlorate. When it was near the demand level, e.g. 0.37 or 0.47 mM, about half of the added

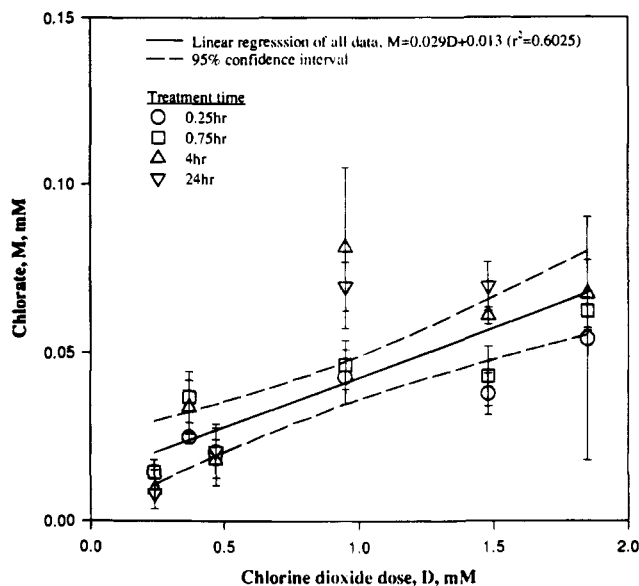


Figure 6. Chlorate in chlorine dioxide treated poultry chiller water. The solid lines represent the linear regression of all data. The error bars indicate 95% confidence intervals of the analysis in triplicate.

Table 3. Percentage of Chlorine Dioxide Reduced to Chlorite and Chlorate in Poultry Chiller Water

chlorine dioxide dose, mM		treatment time				
		0.3 h	0.8 h	4.0 h	24.0 h	72.0 h
1.85	chlorite	18.4	20.0	22.7		43.2
	chlorate	2.9	3.4	3.6		6.0
	total	21.3	23.4	26.3		49.2
1.48	chlorite	20.9	24.3	34.5	49.3	
	chlorate	2.5	2.9	4.1	4.7	
	total	23.5	27.2	38.6	54.0	
0.95	chlorite	39.0	34.0	45.7	58.5	47.9
	chlorate	3.9	4.5	6.4	7.4	
	total	42.9	38.5	52.2	65.9	47.9
0.47	chlorite	51.1	55.3	53.2	66.0	
	chlorate	2.8	2.5	2.4	2.8	
	total	53.8	57.9	55.6	68.7	
0.37	chlorite	45.9	45.9	54.1		56.8
	chlorate	6.7	9.8	9.1		7.4
	total	52.6	55.8	63.1		64.2
0.24	chlorite	70.8	62.5	66.7	75.0	
	chlorate	6.0	6.0	4.0	3.5	
	total	76.8	68.5	70.7	78.5	

chlorine dioxide reduced to chlorite and chlorate. When it was above the demand, the conversion percentage decreased as a result of increasing excess chlorine dioxide. The percentage of conversion appeared to be independent of treatment time when the added chlorine dioxide was less than the demand but to increase with treatment time when the added chlorine dioxide was at or above the demand levels.

In PCW disinfection, the efficiency of chlorine dioxide is reduced by the inorganic and organic materials that compete with microorganisms for the added chlorine dioxide. The efficacy of chlorine dioxide should be inversely influenced by solids content. Chlorine dioxide

demand of PCW will vary not only with solid concentration but also with solid composition because of the variation of reactivity between solids and chlorine dioxide. Chlorine dioxide demand is, therefore, a more precise index than solid content for reflecting the efficacy of chlorine dioxide. To be an effective disinfectant, the chlorine dioxide applied should be near the demand value. Laboratory results with the PCW showed 0.28–0.44 mM chlorine dioxide was sufficient to kill 10^4 cfu/mL aerobic bacteria of natural flora in 3–5 min. This is in agreement with the demand of the PCW at the makeup water inlet. Should 0.30 mM chlorine dioxide be used continuously to disinfect PCW, half of it, or 0.15 mM, will convert to chlorite and chlorate. If each carcass absorbs the maximum PCW that the U.S. regulation allows, 8% of body weight, chlorite and chlorate content in carcass will reach 0.83 mg/kg of chicken. The acceptable daily intake calculated for chlorite as part of a recent food additive petition was reported to be 0.075 mg/kg (Rio Linda Chemical Co., Inc., 1994). This equals 0.09 kg of poultry meats per day per kilogram of body weight. Thus, an adult weighing 70 kg may consume 6.3 kg/day of poultry meats, or four chickens per day, without exceeding the acceptable level of chlorite. This study has shown that it is feasible to control the chlorine dioxide demand of PCW at a reasonable level, for example 20 mg/L, under commercial operating conditions. Disinfecting PCW with chlorine dioxide not only is microbiologically effective but also has a low health risk from the standpoint of chlorite and chlorate contaminations.

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