

Evaluation of Peroxidized Acetic Acid Disinfectant Proper Use Concentration and its Effect on Appearance of Chicken carcasses

ABSTRACT

 With the increase in consumer interest in food safety, in this study, we aimed to investigate the antibacterial effect of 50, 100, 150, and 200 ppm of peracetic acid (peracetic acid A, peracetic acid B, and peracetic acid) and sodium hypochlorite disinfectants on chicken carcasses and contaminated water, respectively, and changes in the appearance of chicken carcasses. Considering the antibacterial effect of each disinfectant concentration, the most significant antibacterial efficacy was observed for general bacteria and *E. coli* at 200 ppm regardless of disinfectant type. Considering the disinfectant type at 200 ppm, sodium hypochlorite was the least effective, and peracetic acid A showed the highest antibacterial efficacy at all concentrations. In chicken carcasses, 200 ppm of peracetic acid A exhibited the highest bacterial reduction rates of 92.7 and 89.3% for general bacteria and *E. coli*, respectively; in contaminated water, 200 ppm of peracetic acid A exhibited a significantly 24 higher reduction rate (p<0.05). Salmonella was negative throughout the experiment, and discoloration of the neck and tip was observed for peracetic acid A and peracetic acid (Daesung) at 100 ppm and peracetic acid B at 150 ppm. Sodium hypochlorite did not cause discoloration at any concentration. Flavor analysis indicated that 100 ppm of peracetic acid A exhibited olfactory characteristics similar to those of 100 or 150 ppm of sodium hypochlorite. In conclusion, 50 ppm of peracetic acid A was adequate for use in poultry processing plants. Keyword: Chicken carcasses, Peroxidized Acetic acid, Sodium hypochlorite, Acetic acid, Octanoic acid.

Introduction

 Many poultry processing plants currently use disinfectants to control microorganisms after slaughter. In particular, sodium hydrochlorite-based disinfectants have most commonly been used for more than 100 years owing to their low cost and high antibacterial efficacy (White, 1998; Northcutt & Jones, 2004; Rutala & Weber, 1997; Hidalgo et al., 2002). However, their disadvantages include the possibility of decreased antibacterial efficacy depending on the environment (Northcutt & Lacy, 2000) and the risk of hypochlorous acid breakdown with decreasing pH of the disinfectant, which can increase the risk of corrosion of equipment and fixtures (Korea Health Industry Development Institute, 2003; European Union, 2017). As presented in Table 1, chlorine-based disinfectants produce toxic chlorine gas when mixed with acids (Fukuzaki, 2006) and react with certain organic substances during the disinfection process to produce the environmental pollutant trihalomethane (THM) (Pavón et al., 2008; Cantor et al., 1978; Morris et al., 1992; Bull et al., 1995; King & Marrct, 1996). Recently, studies have been conducted on disinfectants that can be used safely and effectively as an alternative to chlorine-based disinfectants, with peracetic acid-based disinfectants garnering increasing attention (Kim & Huang, 2020). Peracetic acid (peroxyacetic acid) is a peroxide of acetic acid, produced by making acetic acid react with hydrogen peroxide in the presence of sulfuric acid as a catalyst. At a pH of 5.5-8.2, spontaneous decomposition occurs, primarily by acetic acid and oxygen (Block, 1991; Gehr et al., 2002), wherein acetic acid, hydrogen peroxide, oxygen, and water are produced as decomposition products (Lefevre et al., 1992; Gehr et al., 2002; Wagner et al., 2002). Peracetic acid is a colorless liquid with a pungent vinegar-like odor that is known for its antibacterial properties against a wide range of microorganisms (US Environmental Protection Agency, 2012; Kim & Kim, 2015; Zhang, 2022). In the United States (US), it was approved by the US Food and Drug Administration in 1986 for use as a disinfectant solution

 and subsequently approved by the US Environmental Protection Agency (EPA) and US Department of Agriculture. It is currently used in a variety of industries, including food, medicine, agriculture, alcoholic beverages, institutional horticulture facilities and equipment, animal housing, the dairy industry, and water treatment (Dychdala, 1988; Baldry, 1983; Block, 2001; Kitis, 2004; Luukkonen & Pehkonen, 2017). However, to date, domestic research on the use and appropriate concentration of peracetic acid-based disinfectants in poultry processing plants is limited.

 In this study, we examined the antibacterial efficacy of peracetic acid as a replacement for chlorine-based disinfectants currently used in poultry processing plants; investigated the effect of peracetic acid disinfectant on the appearance of chicken meat by evaluating the quality of chicken meat using an electronic tongue and electronic nose, and established the optimal concentration and safe-use level to meet the food hygiene safety requirements of chicken meat. Among peracetic acid-based disinfectants, there is no difference in the components of samples peracetic acid A and B used in this experiment, but it is thought that applying a small mixture of octane compared to general peracetic acid will protect the chicken's appearance from discoloration compared to peracetic acid and increase the product satisfaction of final consumers This is expected to minimize the spoiled appearance of chicken meat that can occur when using peracetic acid-based disinfectants and improve end- user product satisfaction by preventing industrial hazards, thereby increasing its usability and profitability in the poultry industry.

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Materials and methods

Preparation of sample and materials

 The experimental chickens were Arbor Acres Plus breed and sampled from the Cherrybro poultry processing plant. The contaminated water used for disinfection and verification of

sterilization was mixed with 5 kg of meat and 15 L of water and stored in an incubator at

30 ℃ for 48 h. The deteriorated contaminated water was filtered through a mesh net.

 Peracetic acid was used from Daesung C&S (Oxyacid) as present in Table 2, and the peracetic acid sample was a mixture of peracetic acid (POAA), peroxyoctanoic acid (POOA), hydrogen peroxide, acetic acid, and octanoic acid, as presented in Table 3. The composition of peracetic

acid A and B for the treatment groups was the same. For comparison, 13-15% of

commercially available sodium hypochlorite was used.

Preparing disinfectants

 The disinfectants used in the experiments were prepared, as presented in Tables 4 and 5, and their concentrations were determined by reading the test paper on a dedicated instrument. The tap water used in the experiment was 10 to 15 degrees of water at pH 6 to 7, and the residual chlorine present in the tap water was considered to have no effect on the experimental results. The concentration of each disinfectant was based on the commonly used product (40- 60% acetic acid, 15-20% peracetic + peroxyoctanoic acid, 2.5-10% hydrogen peroxide).

Applying disinfectants to carcasses

 At each concentration of the four disinfectants, 21 carcasses were immersed for 5 min (based on the time required to pass through the combination chiller during the conventional poultry processing process) and subsequently placed in a refrigerator below 5 ℃ for 1 h (based on the time required to pass through the air chiller for 1 h during the conventional poultry processing process), and the test was conducted according to the bacteriological test method for meat according to the Food Code.

Applying contaminated water to carcasses

 We collected contaminated water 12 times (10 mL each) to be used as raw samples. The experimental samples were prepared by creating 321 samples of 9 mL of raw contaminated water samples and dispensing 1 mL of each concentration in four disinfectants (peracetic acid (Dae sung), peracetic acid A, peracetic acid B, and sodium hypochlorite), diluting them with a vortex mix for 30 s, and subsequently vortexing for 30 min. For *Salmonella*, 22.5 mL of raw contaminated water sample was prepared, treated with four disinfectants (peracetic acid, peracetic acid A, peracetic acid B, and sodium hypochlorite) at 50, 100, 150, and 200 ppm each in a 2.5-mL aliquot (applied by 10%), diluted with a vortex

mixer for 30 s, and stabilized for 30 min prior to use.

Experimental methods

 For the general bacterial count experiment, the experimental solution was re-homogenized with a vortex mixer, and the samples were taken in 1 mL aliquots with a micropipette and 122 diluted in 9 mL of 0.85% sterile PBS to concentrations of 10^4 , 10^5 , and 10^6 ; subsequently, they were incubated in a general dry-film medium to measure the bacterial count. The resulting red colonies were counted and multiplied by the dilution factor to determine the general bacterial count. The reduction rate (%) calculated dividing (Initial bacterial count – Count of bacteria after 10 minutes) by initial bacterial count and multiplying 100. For the count experiment of *E. coli*, the dilutions prepared the same way as those for the general bacterial count experiment were incubated on *E. coli* dry-film medium, and the bubbles formed around the colonies after incubation were counted and multiplied by the dilution factor to determine the *E. coli* count. The Salmonella test was conducted by adding sterilized buffered peptone water (BPW) to the prepared test solution for primary growth, and the culture was harvested and sub-cultured in Rappaport-Vassiliadis (RV) medium for

 secondary growth. The cultures from the second round of growth were then sub-cultured onto xylose lysine deoxycholate (XLD) agar and Brilliant Green (BG) Sulfa Agar, with XLD agar and BG Sulfa Agar being considered positive when black and red colonies occurred, respectively, and the test was finally confirmed to be positive when all media showed positive results. The reduction rate (%) calculated dividing (Initial bacterial count – Count of bacteria after 10 minutes) by initial bacterial count and multiplying 100.

139 Heracles II Electronic Nose (Alpha MOS, Toulouse, France) was used to analyze the flavor components of the samples, and the measurement results were expressed as the rate of change 141 of the resistance value of the volatile components (R_{gas}) of the samples with respect to the resistance value of air (Rair) using Alpha Soft software (Alpha MOS, Toulouse, France) for flavor principal component analysis (PCA); the sensitivity of each sensor was expressed as 144 delta (R_{gas}/R_{air}). The measured flavor components were represented in a PCA plot, and the first (PC1) and second principal component (PC2) values were obtained to distinguish the flavor patterns. For comparison of peracetic acid and sodium hypochlorite acid, set peracetic A as control and sodium hypochlorite acid as treatment. (C-100 = peracetic A 100ppm; C-150 148 = peracetic A 150ppm; T-100 = sodium hypochlorite acid 100ppm; T-150 = sodium hypochlorite acid 150ppm)

Statistical processing

 All experiments were conducted with at least three replicates and the results were expressed as the mean and standard deviation. Statistical analysis was conducted using Minitab 18 (Minitab Inc.). One-way analysis of variance (ANOVA) was used to test the significance (p<0.05) of each sample, and Tukey's multiple range test was used for the post-hoc test.

Results and discussion

Antibacterial efficacy by disinfectant concentration

 Table 6 presents the antibacterial efficacy of peracetic acid (Daesung) on carcasses and contaminated water. The reduction of general bacteria in the carcasses was not significantly different at 50, 100, and 150 ppm but tended to be the lowest (60.2%) at 100 ppm. At 200 163 ppm, the bacterial count significantly reduced from 5350.0 before treatment to 388.5 after treatment (p<0.05). For E. coli, no significant differences were observed, with reduction rates of 63.8 and 66.7% at 50 and 100 ppm, respectively, but E. coli decreased significantly by 71.3 and 89.3% at 150 and at 200 ppm, respectively (p<0.05). When applied to contaminated water, the highest and lowest decreases in the number of general bacteria were 63.5 and 46.5% at 200 and 50 ppm, respectively (p<0.05). Similar to general bacteria, E. coli showed 169 the highest reduction at 200 ppm, with an 82.4% reduction from 3.6×10^7 to 6.3×10^6 , but significance was not identified.

 Table 7 presents the antibacterial efficacy of peracetic acid A on carcasses and contaminated water. When applied to carcasses, the largest decrease in the number of general bacteria in contaminated water was 98.4% at 200 ppm, whereas the reduction rate was 174 significantly lower (88.8%) at 50 ppm (p<0.05), showing no significant differences at other concentrations. For E. coli, no significant difference was observed at all concentrations, but the lowest reduction rate was 91.6% at 50 ppm, and the antibacterial efficacy tended to increase in a concentration-dependent manner. When applied to contaminated water, general bacteria decreased by 58.6% at 50 ppm, 64.3% at 100 ppm, and 72.8% at 150 and 200 ppm, showing a significantly higher antibacterial efficacy (p<0.05). For E. coli, the antibacterial 180 efficacy was the highest at 200 ppm, with a reduction in the count of E. coli from 3.6×10^{7} to 181 2.7×10⁶ (p<0.05), followed by those at 100 (88.0%) and 150 ppm (84.0%), with no significant

difference between them; 50 ppm of peracetic acid A showed the lowest reduction rate,

183 namely, 79.1% (p<0.05).

 Table 8 presents the antibacterial efficacy of peracetic acid B on carcasses and contaminated water. When applied to carcasses, the reduction in general bacteria was lowest at 50 ppm, with no significant difference from that at 100 ppm. The highest reduction was 187 observed at 200 ppm, with a significant reduction of 92.5% (p<0.05). For E. coli, the largest reduction was 92.2% at 200 ppm (p<0.05), followed by 85.0% at 150 ppm, and no significant reduction at 100 and 50 ppm. When applied to contaminated water, the bacterial reduction was higher in general bacteria with increasing disinfectant concentration, but no significant difference was observed between them. For E. coli, the largest reduction was 82.9% at 200 192 ppm, and the reduction rate was significantly lower (61.4%) at 50 ppm ($p<0.05$), with no significant difference between concentration of 100 and 150 ppm. Table 9 presents the antibacterial efficacy of sodium hypochlorite on carcasses and contaminated water. When applied to carcasses, the antibacterial efficacy was significantly higher at 200 ppm (78.3%; p<0.05), followed by those at 150 and 100 ppm; it then decreased to 47.3% at 50 ppm. For E. coli, the largest reduction was found at 200 ppm (p<0.05), and the antibacterial efficacy decreased in a concentration-dependent manner, but no significant difference was observed among them. When applied to contaminated water, the largest decrease in the number of general bacteria was 56.3% at 200 ppm, and the lowest reduction 201 rates were 29.4 and 35.0% at 50 and 100 ppm, respectively ($p<0.05$). The reduction rates for E. coli were 56.3, 48.3, 35.0, and 29.4% at 200, 150, 100, and 50 ppm, respectively, with no

significant differences between those at each concentration.

 Referred to results of table 6-9, based on the results in section 200 ppm was set as the optimal concentration for each disinfectant in this study. The comparison of the antibacterial efficacy of each disinfectant at the optimal (200ppm) concentration is presented in Table 10.

 Before applying disinfectant to treatment, all treatment have no statistically significance in result of antibacterial efficacy. All disinfectants except sodium hypochlorite showed a 209 bacterial reduction rate of 90% when applied to carcasses ($p<0.05$). In particular, when applied to carcasses, peracetic acid A showed a significant reduction of 99.4% in E. coli levels from 6941.7 before treatment to 44.2 after treatment compared with that in the control (p<0.05). When applied to contaminated water, peracetic acid A showed the highest significant reduction among all disinfectants, with a reduction rate of approximately 80% (p<0.05). However, no significant difference was observed in antibacterial efficacy between peracetic acid (Daesung) and peracetic acid B. The average reduction from the control was the highest for peracetic acid A, peracetic acid B, peracetic acid (Daesung), and sodium hypochlorite, with sodium hypochlorite showing the lowest reduction among all disinfectants, 218 regardless of concentration $(p<0.05)$. The tests of antibacterial efficacy on sample carcasses revealed that the peracetic acid

 series had higher antibacterial efficacy than sodium hypochlorite at the same concentration. This result is consistent with the trends observed in other previous studies (Kim et al., 2010; Lee et al., 2006; Lee, 2020). Considering the peracetic acid series, peracetic acid A showed an antibacterial efficacy of more than 90% at 50 ppm and a reduction rate consistently exceeding 90% at other concentrations, which are considered to be the highest among all 225 disinfectants $(p<0.05)$.

 The antibacterial efficacy tests on contaminated water revealed that the peracetic acid- based disinfectants had a significantly higher reduction rate than sodium hypochlorite at the 228 same concentration $(p<0.05)$. When comparing peracetic acid-based disinfectants, peracetic acid A had the highest reduction rate at all concentrations, distinguishing it from the other 230 disinfectants ($p<0.05$), whereas peracetic acid B and peracetic acid (Dae sung) had similar effects.

The effect of each disinfectant on the appearance of chicken The changes in the appearance of chicken are shown in Figures 1 to 4. Discoloration was observed on the neck and tips with peracetic acid and peracetic acid A at 100 ppm and peracetic acid B at 150 ppm, whereas no discoloration was observed with sodium hypochlorite at any concentration. Meat color are subjective characteristic of meat that perceived by consumer. And, consumers tend to favor chicken meat that closely resembles the color of the meat they typically consume (Manjankattil et al., 2021). Various organic acids have been studied for application in poultry processing plant including acetic, citric, and lactic acid. (Mulder et al., 1987; Dickens et al., 1994). It has been reported that these acids, while effective as antimicrobials, may result in negative flavor and color alterations (Blankenship et al., 1990). In current experiment, discoloration was observed on the neck and tips with peracetic acid and peracetic acid A at 100 ppm and peracetic acid B at 150 ppm. However, no discoloration was observed with sodium hypochlorite at any concentration. These results disagree with Bauermeister et al. (2008), as there were no differences in the lightness values of the 0.01% and 0.015% peracetic acid levels and sodium hypochlorite. The reason for these inconsistent results in appearances may be due to the different analysis methods of meat color. In our experiment, we simply analyze changes in appearances, therefore, a precise analysis method 251 is needed for further study such as Hunter $L^*a^*b^*$ color system. **Analysis results of** *Salmonella Salmonella* was not detected in all samples at each concentration, as presented in Table 11.

Electronic nose analysis results

 Figure 5 shows the PCA results of the electronic nose. In the PCA section of the sample, the values of PC1 and PC2 were 99.992 and 0.005517%, respectively, and the differences between treatments were mainly distinguished by PC1. Along the x-axis, C-100, T-100, and T-150 did not show a significant change in position among treatment groups, with C-150 being the furthest to the right and clearly distinguishable from the other treatment groups. C- 100, T-100, and T-150 seemed to exhibit similar flavors, whereas C-150 exhibited a different flavor profile from the other treatment groups. Therefore, the olfactory characteristics after disinfection with sodium hypochlorite at 100 or 150 ppm is expected to be similar to those after disinfection with peracetic acid A at 100 ppm.

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Conclusions

 In this study, we evaluated the antibacterial efficacy of three peracetic acid-based disinfectants and a sodium hypochlorite disinfectant applied to carcasses and contaminated water to determine the effect of peracetic acid on chicken meat. In the results of antibacterial efficacy tests, peracetic acid-based disinfectants had a significantly higher reduction rate than sodium hypochlorite. Increasing concentration of peracetic A had higher reduction rate than others at the same concentration. However, discoloration was observed on the neck and tips with peracetic acid A at 100 to 200. In conclusion, considering both reduction rate of bacteria and appearance, 50ppm of peracetic acid A was adequate for use in poultry processing plants.

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414 **Table 1. By-products after disinfection**

1) Florida Department of Environmental Protection surface water limit for Class III marine waters

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417 **Table 2. Peracetic acid product information**

420 **Table 3. Preparation of the peracetic acid mixtures**

Table 4. Preparation of the peracetic acid disinfectants

1) The tap water was 10 to 15 degrees of water at pH 6 to 7

Table 5. Preparation of the sodium hypochlorite disinfectant

1) The tap water was 10 to 15 degrees of water at pH 6 to 7

Classification			50ppm	100ppm	150 ppm	200 ppm	SEM	p-value
Before treatment	Carcasses	General bacteria	5350	5350	5350	5350	13.4	0.98
		E. coli	925.8	925.8	925.8	925.8	18.74	0.97
	Contaminated	General bacteria	3.6×10^8	3.6×10^8	3.6×10^{8}	3.6×10^8	1.4×10^{7}	0.97
	water	E. coli	3.8×10^{7}	3.6×10^{7}	3.6×10^{7}	3.6×10^{7}	1.7×10^{6}	0.98
After treatment	Carcasses	General bacteria	1731.5^{b}	2127.5^b	980.5^{b}	388.5^{b}	415.06	0.06
		E. coli	335.0^b	308.5^{b}	266.0^{b}	98.6^b	60.28	0.08
	Contaminated	General bacteria	1.9×10^{8} b	1.7×10^{8} b	1.5×10^{8} ab	1.3×10^{8} ^a	1.71×10^{8}	< 0.05
	water	E. coli	9.7×10^{6} a	8.7×10^{6} ab	6.8×10^{6} ^a	6.3×10^{6} c	1.8×10^{5}	< 0.05
Redution rate $(\frac{9}{6})^{2}$	Carcasses	General bacteria	67.6^{b}	60.2^{b}	81.7^{b}	$92.7^{\rm a}$	17.46	< 0.05
		E. coli	63.8°	66.7°	71.3^{b}	89.3 ^a	11.46	< 0.05
	Contaminated	General bacteria	46.5^{b}	52.4^{b}	58.5^{ab}	$63.5^{\rm a}$	7.37	< 0.05
	water	E. coli	74.5^{b}	75.7^{ab}	81.1 ^a	82.4°	3.92	< 0.05

Table 6. Antibacterial efficacy of peracetic acid (Daesung) on carcasses and contaminated water1)

1) Each values are mean ±SD of at least three repeated experiments.

2) Redution rate(%) : (Initial bacterial count – Count of bacteria after 10 minutes)/Initial bacterial count*100

 $\sum_{i=1}^{n}$

Classification			50ppm	100 ppm	150 ppm	200 ppm	SEM	p-value
Before treatment	Carcasses	General bacteria	18816	18816	18816	18816	19.7	0.99
		E. coli	6941.7	6941.7	6941.7	6941.7	41.45	0.96
	Contaminated	General bacteria	3.6×10^8	3.6×10^8	3.6×10^8	3.6×10^8	1.4×10^{7}	0.97
	water	E. coli	3.8×10^{7}	3.6×10^{7}	$3.6\times10'$	3.6×10^{7}	1.7×10^{6}	0.98
After treatment	Carcasses	General bacteria	2113.0^{b}	1110.5°	884.0^{b}	292.0^{b}	288.26	< 0.05
		E. coli	585.5^{ab}	139.0 ^b	$122.0^{\rm b}$	44.2^{b}	56.98	< 0.05
	Contaminated	General bacteria	1.5×108 b	1.3×10^{8} b	9.8×10^{7}	9.8×10^{7} a	1.6×10^{7}	< 0.05
	water	E. coli	7.9×10^{6} a	4.3×10^{6} b	5.8×10^{6} ^a	2.7×10^{6} b	3.4×10^6	< 0.05
Redution rate $(\frac{9}{6})^{2}$	Carcasses	General bacteria	88.8 ^c	94.1 ^b	95.3^{b}	98.4°	1.46	< 0.05
		E. coli	91.6^{bc}	98.0^{b}	98.2^{b}	99.4^{b}	3.53	< 0.05
	Contaminated	General bacteria	58.6^{b}	64.3^{b}	72.8 ^a	72.8 ^a	6.92	< 0.05
	water	E. coli	79.1 ^c	88.0^{b}	84.0 ^b	$92.4^{\rm a}$	5.66	< 0.05

Table 7. Antibacterial efficacy of peracetic acid A on carcasses and contaminated water1)

1) Each values are mean ±SD of at least three repeated experiments.

1) Each values are mean ±SD of at least three repeated experiments.

Table 9. Antibacterial efficacy of sodium hypochlorite on carcasses and contaminated water1)

1) Each values are mean ±SD of at least three repeated experiments.

Table 10. Comparison of antibacterial efficacy at the optimal concentration1)

1) Each values are mean ±SD of at least three repeated experiments.

Table 11. *Salmonella* **test results**

1) $N = Negative$

Figure 1. Discoloration of chicken meat by peracetic acid (Daesung) at each concentration

- * The changes in the appearance of chicken after leaving in conductors in disinfectant for 1 hour
- * Discoloration was observed on the neck and tips at 100 ppm

* The changes in the appearance of chicken after leaving in conductors in disinfectant for 1 hour

* Discoloration was observed on the neck and tips at 100 ppm

Figure 2. Discoloration of chicken meat by peracetic acid A at each concentration

* The changes in the appearance of chicken after leaving in conductors in disinfectant for 1 hour

* Discoloration was observed on the neck and tips at 150 ppm

Figure 3. Discoloration of chicken meat by peracetic acid B at each concentration

* The changes in the appearance of chicken after leaving in conductors in disinfectant for 1 hour * no discoloration was observed at any concentration.

Figure 4. Discoloration of chicken meat by sodium hypochlorite at each concentration

Figure 5. Principal component analysis (PCA) results of chicken skin treated with sodium hypochlorite and peracetic acid A by

concentration. *C-100: peracetic acid A, 100 ppm; C-150: peracetic acid A, 150 ppm; T-100: sodium hypochlorite, 100 ppm; T-150: sodium hypochlorite, 150 pp