

**CENTER FOR PRODUCE SAFETY
FINAL REPORT**

Project Title: Establishment of Critical Operating Standards for Chlorine Dioxide in Disinfection of Dump Tank and Flume Water for Fresh Tomatoes

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Industry Collaboration: AquaPulse Systems
Tomato Packing Cooperators, FL and CA

Executive List of Key Outcomes:

MODEL SYSTEM

- ClO₂ was confirmed to be effective in achieving a 6-log reduction within 2 min under a range of water quality and temperature conditions.
- Water quality strongly affected the rate of inactivation
- Salmonella serotype strongly affected the rate of inactivation
- ORP (mV) and ClO₂ ppm tracked well in ‘clean’ water but less well as water constituent complexity increased
- 5 ppm ClO₂ is a BMP setpoint for water quality that exceeds 50 FAU

COMMERCIAL SURVEY

- ClO₂, alone, which is limited to 5 ppm by regulatory standards, is unlikely to meet BMP goals for dump tank management over typical commercial conditions.
- ClO₂ at or between 3-5 ppm will meet BMP goals for flume water management over typical commercial conditions. Lower doses may be adequate if flume water is maintained at a low oxidative-demand condition.
- Accurate colorimetric measurement of residual ppm of ClO₂ was not possible in high turbidity dump tank water without further processing due to background interference.
- Maintenance of BMP doses of ClO₂ will maintain or significantly reduce microbiological loading of the water but are difficult to achieve under current practice.

INTRODUCTION

The genesis of this project was a direct response to and result of discussions among industry associations, representatives of the full fresh tomato supply chain, public health regulators, government and private auditors, and academia during the development of Tomato Food Safety Audit Protocol (<http://www.unitedfresh.org/>). The general lack of performance data for specific postharvest water sanitizers currently in use or being adopted and developed under conditions reflective of commercial systems was identified as an obstacle to setting meaningful standards. Performance ‘metrics’ were needed to build consensus around these audit criteria in a manner that would advance tomato food safety goals in both business integrity and consumer protection.

The overall objective of this bicoastal project was to develop scientifically-based critical operating standards for chlorine dioxide use in dump tank and flume tank waters for the fresh tomato industry. A two-prong approach was developed through an iterative process of model system assessments and on-site surveys of post-fruit-contact water quality and incoming and post-wash-process fruit. The on-site assessments allowed defining of specific parameters for water temperature, chlorine dioxide dose and water turbidity. These parameters were utilized to adjust the parameters of a model tomato dump-flume water for *in vitro* experiments. This ‘synthetic’ process water was further utilized to determine correlations among chlorine dioxide concentration, water turbidity and temperature as well as rates of *Salmonella enterica* inactivation suspended in this sanitizer-conditioned water. In commercial operations, measurements included microbiological enumeration, chlorine dioxide concentration and physicochemical parameters (oxidation reduction potential (ORP), turbidity, conductivity and temperature) in water dump tanks and flume systems. Cooperating tomato facilities in Florida (Facility A and B) and California (Facility C) running with matched chlorine dioxide generation/injection systems for all water contact units were visited on four dates each during their respective seasons.

The anticipated outcome was to develop data-based Best Management Practices (BMPs) guidance for chlorine dioxide treatment of process water used in the fresh tomato industry that would be applicable to primary packers, re-packers, and fresh processors. The work plan was further designed to result in outcomes and BMPs that would be reasonably transferable to other commodities with similar water quality management challenges and safety performance expectations.

OBJECTIVES

Objective 1: Conduct on-site assessments of chlorine dioxide dose management and quantitative microbiological water quality in commercial dump tank and flume systems within commercial tomato packing operations in Florida and California.

Objective 2: Determine the comparative correlative capacity of oxidation reduction potential (ORP; mV) vs. dose (mg/L; ppm) to monitor, control, and document water disinfection status within commercial tomato packing operations in Florida and California.

METHODOLOGY

Tomato packing facilities

Dump and Flume System Schematic

Diagrams of each operational system, the associated process flow, and sampling points are provided in Figures 1-3 below

Tomato

At each tomato facility, 10 tomatoes were collected every 30 min during a period of 5.5 hours. The sampling points were at the top and bottom of the field transport gondola (Top and Bottom) and at the point tomatoes were conveyed out of the flume system and before grading or worker contact (After Washing). Tomatoes were collected in a sterile plastic bag and clean disposable gloves were changed between each sampling point and between each sampling location to avoid cross contamination. For each sampling point 5 tomatoes were utilized to determine on-site pulp temperature of the fruit which was done with a portable produce temperature probe. The probe was calibrated in an ice-water slurry before each sampling date. The temperature was measured for each tomato at the stem scar region (~1cm deep). The remaining 5 tomatoes from each point were used to quantify viable populations of total aerobic mesophiles, total coliforms and *E. coli*. Tomatoes utilized for microbiological analysis were transported to the laboratory inside a cooler and stored at 2.5°C for about 14 hours. Tomatoes were removed from storage and individually placed in 100 mL of 0.1% sterile buffered peptone water (BPW) and vigorously rubbed to remove attached bacteria. Cell suspensions were diluted and plated on Plate Count Agar to determine total mesophilic population and on CHROM-ECC (ECC) to determine total coliforms and *E. coli* populations. Plates were incubated for 24 hours at 30 and 37°C, for mesophiles and coliforms respectively. Each sampling point and location was repeated twice.

Dump tank and Flume Water

Water samples were taken from dump tank (Tank 1A) and flume system (Tank 2A) and their respective re-circulation system tanks (Tank 1B and Tank 2B, respectively), for a total of four samples every 30 min. Water samples were taken to determine microbiological content and to assess physicochemical parameters. For microbiological enumeration a 100 mL of water sample was taken in duplicate and immediately neutralized using sodium thiosulphate. For experiments performed at tomato facilities in Florida (A and B), the sodium thiosulphate was in tablets previously pulverized to facilitate dissolution, but preloaded to sampling containers in a liquid form (1N) on trials performed in California. In both cases the amount of thiosulphate utilized was in excess to ensure neutralization of the sample and this amount, as well as the use of the aqueous form, was previously tested in the laboratory to ensure efficacy. Samples were immediately placed on ice and transported to respective laboratories and analyzed within 14 hours. Water samples were diluted and plated on PCA and ECC to determine total aerobic mesophilic and coliform populations as described above. For trials 3 and 4 performed in California, the population of coliforms and *E. coli* was determined utilizing the QuantiTray Colilert system (Idexx Laboratories). This protocol change was done after observing very low or no detectable populations on ECC plates. The QuantiTray format permitted us to easily test a sample size of 100 mL in contrast with spread plating ECC that was limited to 1 ml (composite

of four replicate plates @ 250 µl each). For all trials, populations of *E. coli* and coliforms were normalized to 100 mL of sample to facilitate comparison. Each sampling point and location was repeated twice.

A separate water sample of approximately 500 ml was taken at each sample point to determine the oxidation reduction potential (ORP), pH, water temperature, conductivity, chlorine dioxide concentration and turbidity using standard protocols and matched analytical instruments between the UFL and UCD research teams. Specifications of the instruments used in this study are provided in Table 1. During the sampling at tomato facility in California it was apparent that conditions of elevated turbidity interfered with the accuracy of the method employed to determine chlorine dioxide concentration. At high turbidities (>100 FAU) the concentration detected in the chlorine dioxide meter was higher than the real value due to the ability of the dissolved and suspended constituents in water to absorb light at the same wavelength used to determine ClO₂ concentration prior to adding reagents to obtain a ppm-reading. For this reason during trials 3 and 4, samples were filtered using a 0.45 µm membrane to clarify the sample and avoid interference. The validity of filtration was determined under laboratory conditions. Each physicochemical parameter and each sampling point and time was repeated three times.

FACILITY A (FLORIDA)

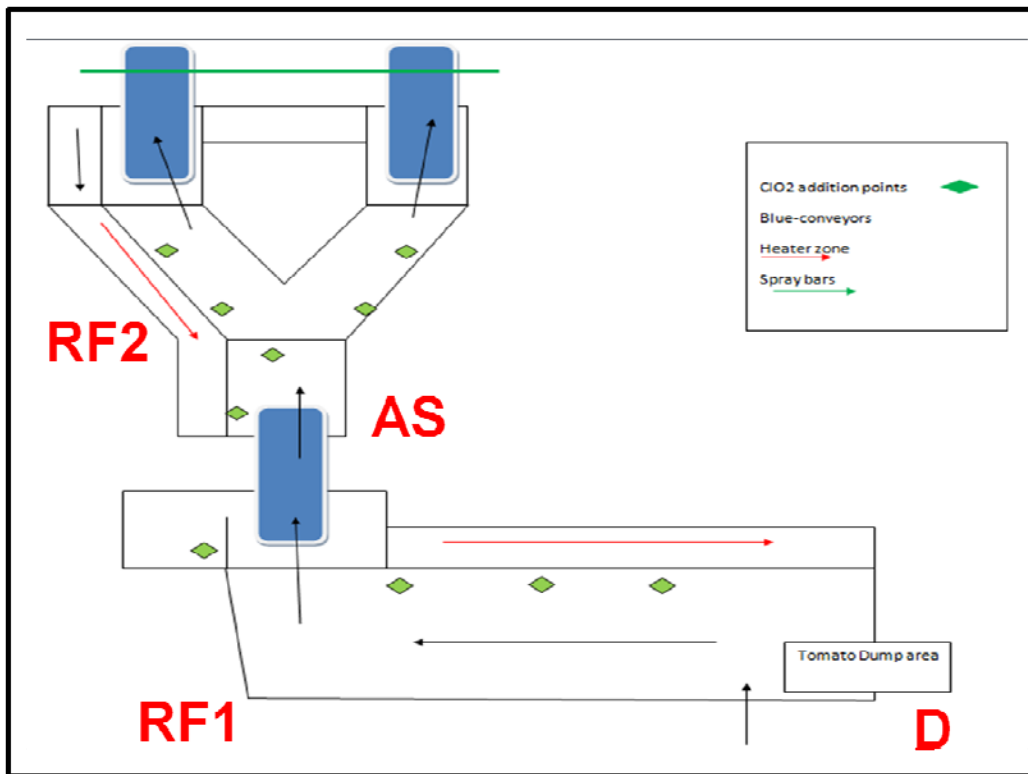


Fig. 1- South Florida schematic layout of tomato dump and flume tank water. Water operations for sample collection are: D = Dump, RF1 = End of the 1st flume, AS = Beginning of the 2nd flume and RF2 = End of the 2nd flume.

FACILITY B (FLORIDA)

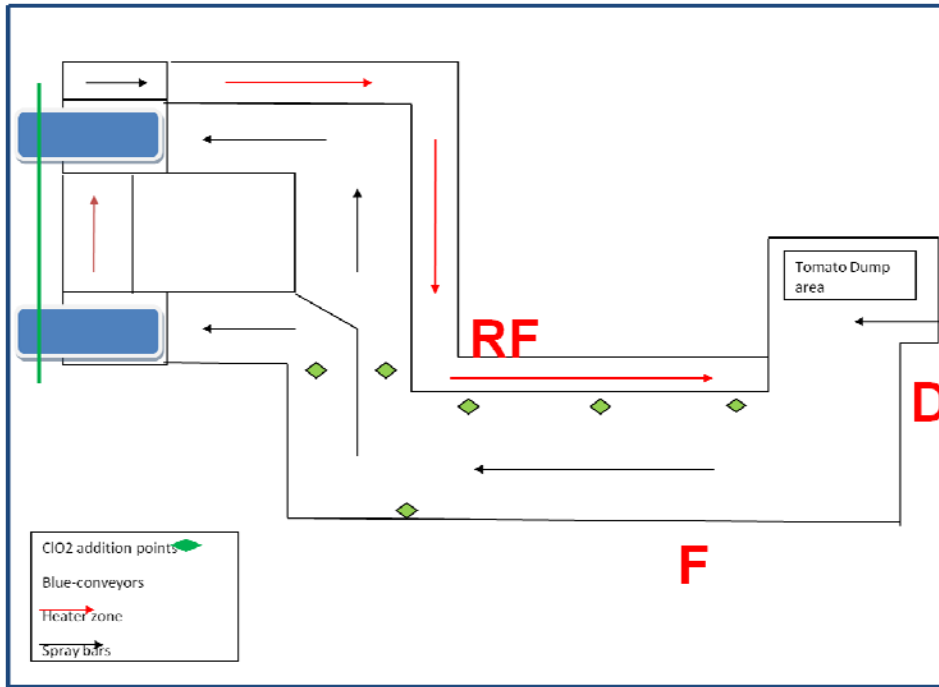


Fig. 2 - Central Florida schematic layout of tomato dump and flume tank water. Water operations for sample collection are: D = Dump, F = Beginning of the 1st flume and RF = End of the 1st flume.

FACILITY C (CALIFORNIA)

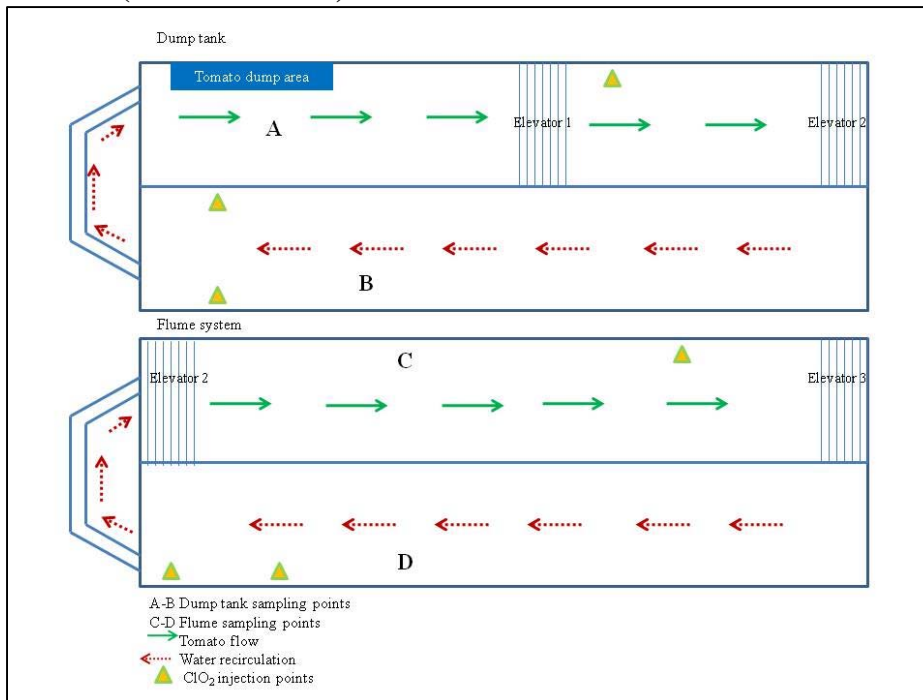


Fig. 3 – Central California schematic layout of tomato dump and flume tank water.

Table 1. Instrumentation for Water Analysis Used in CA and FL Studies

Measurement	Quantitative Method	Supplier Model
Water Temperature	Portable probe	Thermo-Russell – RL060P
Fruit Pulp Temperature	Digital	QA Supply - 06E1
Air Temperature	Digital	QA Supply - 06E1
pH	Temperature compensated sensor	Thermo-Russell – RL060P
Turbidity	FAU	Hach – DR/850
Conductivity	EC – $\mu\text{S}/\text{cm}$ (1-1999)	Hanna – DiST WP3
ORP	Redox – millivolts Temperature compensated sensor	Thermo-Russell – RL060P
ClO ₂ residual	Colorimeter – 0-3.8 ppm	Orbeco Hellige – SC400
ClO ₂ residual	Colorimeter – 0-3.8 ppm	Hach Pocket II

Model System Experimental Design

To determine the influence of physicochemical parameters on inactivation of *Salmonella enterica*, a model tomato dump-flume water (synthetic water) was designed to reproduce a consensus composition based on constituent analysis of water sampled at six CA tomato facilities during a previous survey of hypochlorite performance (Suslow report to CA Tomato Commission; 2005). To further simulate the background oxidative-demand of dump and flume water, tomato plants with adhering soil were taken from a tomato planting located at UCD and submerged in water. Following coarse filtering, the water infusion was adjusted to turbidity values of 22, 43 and 160 FAU. Synthetic water was autoclaved to facilitate enumeration of inoculated *Salmonella*.

Table 2: Compositional Analysis of Commercial Tomato Dump-Flume Water

pH	EC [SOP 815] dS/m	Na (Soluble) [SOP 835] meq/L	Cl [SOP 830] meq/L	SO ₄ -S [SOP 860] mg/L	NO ₃ -N [SOP 847] mg/L	HCO ₃ [SOP 820] meq/L	Zn (Soluble) [SOP 835] mg/L	Cu (Soluble) [SOP 835] mg/L	Mn (Soluble) [SOP 835] mg/L	Fe (Soluble) [SOP 835] mg/L	TSS [SOP 870] mg/L
7.5	3.3	28.6	21.4	35.8	6.8	5.4	0.2	0.3	0.3	0.7	96.2

Baseline model water composition was derived from the mean values of dump and flume tank water obtained from six California tomato packing operations in previous surveys. All analyses were performed by the UC ANR Analytical Services Lab (<http://danranlab.ucanr.org>) using SOP methods associated with each column. The method for pH determination was SOP 805. The range of Total Suspended Solids (TSS) was 8 to 536 which fluctuated with incoming fruit loads and periodic fresh water exchange but invariably increased at each facility over the course of daily operation.

A 3x3x3 factorial experiment was designed to assess the effect of water turbidity (three levels: 22, 43 and 160 FAU), water temperature (three levels: 10, 25 and 40°C) and ClO₂ concentration (three levels: 1, 3 and 5 mg/L) in the inactivation of *S. enterica* sv Newport which was previously involved in a tomato outbreak (total of 27 treatments). Five mg/L is the maximum allowable concentration for direct contact with fresh produce. Additionally the same factorial design was utilized to determine the behavior of ORP, pH and residual ClO₂ in the system. All these experiments were performed in total volume of 100 ml and for a period of two minutes.

For each experiment, each 100 ml of synthetic water was adjusted to the desired temperature (10, 25 or 40°C) and 10^7 cells of *S. enterica* sv Newport (Rifampicin resistant strain PTVS 077) were added to the system. After homogenous distribution of the inoculum, ClO_2 , from a concentrated stock solution, was added to reach 1, 3 or 5 mg/l. At time-points of 5, 10, 15, 30, 45, 60, 75, 90 and 120 sec a 1 ml of sample was taken and placed in a tube containing 9 ml of Dey/Engley neutralizing broth. The contents of each tube was either diluted or directly plated on Tryptic Soy Agar supplemented with 50 mg/L rifampicin to determine the log reduction of *S. enterica* for each treatment. Additionally tubes were incubated at 37°C for 24h to establish an enrichment-based Presence/Absence test for any timepoint giving negative results by direct enumeration. Enrichment allowed qualitative determination of the contact time needed for inactivation of *S. enterica* within the test system. Each treatment was repeated three times.

In separate test without pathogen inoculation, the same system was prepared to determine the change in ORP, pH and ClO_2 under the same conditions of temperature and water turbidity, for a period of 120 sec, after addition of 1, 3 or 5 mg/L of ClO_2 . Determination of these physicochemical parameters was performed using the same standard protocols utilized at the tomato facilities.

In addition, this experiment was repeated for all conditions of turbidity and ClO_2 concentration but only at 25°C for six different *S. enterica* strains. Also, the experiments for *S. enterica* sv. Newport were compared for all the conditions but only at 25°C with three different concentrations of sodium hypochlorite (5, 25 and 50 mg/L) adjusted to pH 7.0.

RESULTS AND KEY OBSERVATIONS

Tomato Facility Surveys

A complete set of figures for all tomato packing facility water quality and microbiological analyses are provided in the Appendix.

Overall Assessment of Water Quality Management: *Chlorine dioxide can be managed as a water treatment sanitizer for tomato flume and spray-wash systems but current operational limitations greatly restrict its efficacy in typical dump tank management. Current standards in the Tomato Food Safety Audit Protocol should be modified to reflect this reality. Modifications to enhance the management of dump tank systems where application of ClO_2 is desirable for an individual operation remains a reasonable approach.*

- A positive linear correlation was determined between ORP (mV) and ClO_2 (ppm) [Figure 4] and ORP and Turbidity (FAU) [Appendix Fig. 28 – flume water only] as well as Turbidity and ClO_2 in both, dump and flume tanks. Despite better outcomes in in flume water systems, the strength of the relationship between ClO_2 and ORP appears too be insufficient to rely on ORP sensors, alone, to predictably control microbiological water quality in real-world systems involving this first-stage tomato supply chain point.
- When comparing linear correlations between ORP with turbidity and conductivity, negative correlations were determined in the dump tank, however in the flume system the correlation for these parameters was positive.
- All analyses for statistical correlation demonstrated a tendency between the relationship of all physicochemical parameters evaluated, however while the assessment of normality

for data distribution was determined to be valid, the linear correlations were not valid according to Durbin-Watson statistic test.

Facility Outcomes and Observations

It is important to point out that these studies represent both a longitudinal and cross-sectional performance baseline for limited set of tomato packing operations in a general sense and with respect to their adoption of a single source chlorine dioxide generation and dose-management. We feel both industry cooperators should be acknowledged and commended for their willingness to allow objective data-gathering for public distribution and scrutiny. We believe the project outcomes, beyond the focus on efficacy of a single water treatment, can be broadly applied to the continual internal and proactive efforts of the fresh tomato industry leadership to improve food safety standards and performance.

Some key general outcomes include;

- Differences in microbial content of dump vs. flume water can be large (> 3 -log) but may also be minimal (Table 3)
- Differences in microbial content between incoming and washed fruit (within the limits of sampling for this project) were generally low and a gain in mean bacterial counts was occasionally observed (Table 4).
- The temperature differential between incoming fruit and dump tank or flume water was difficult to manage at any moment in daily operations but well managed overall (Appendix Fig 6 and 20).
- Mean fruit pulp temperature exiting the process line was not consistently warmer than entering it (Table 5).
- There were periods of time where ORP in the flume systems was <600 mV. ORP in the flume system was often >600 mV, however measured concentrations of chlorine dioxide were above 5 mg/L, exceeding the approved limit.
- Total mesophiles in water were always larger in dump tank than in flume tank and total coliforms were mostly detected only in dump tank but not in flume tank.
- In the first two trials *E. coli* was not detected in the flume tank, but it was detected in the dump tank mostly within the first hour of operation, it is important to mention that ECC was employed for *E. coli* detection. For CA trials 3 and 4 quantification of total coliforms and *E. coli* was assessed using QuantiTray system. For these two trials coliforms were detected for both dump tank and flume system and *E. coli* was detected in the dump tank during the entire period of sampling in both trials.

It is important to point out the tremendous variation in the results among each trial result of different environmental conditions and conditions of the incoming fruit which can significantly alter the conditions. It is obvious that the flume system is able to maintain a value of ORP >600 mV, which according to the current guidelines should be able to inactivate vegetative cells including foodborne pathogens like *Salmonella enteric* and related enteric bacterial pathogens.

With the results obtained for the fruit, it is evident that there is not a significant reduction in the bacterial load, although it is unknown the composition of the microorganisms present at the end

of the washing process, it is evident that in several points the tomatoes tend to increase in microbial load.

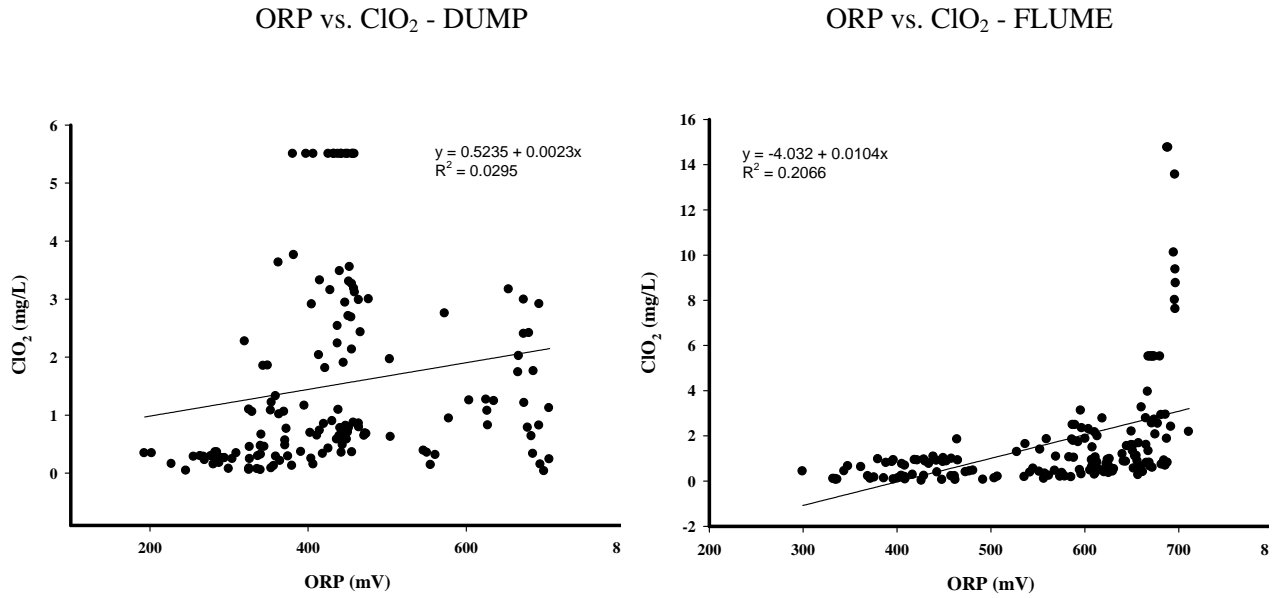


Figure 4. – Scatter diagram representation of correlation between measured oxidation reduction potential (mV) and chlorine dioxide (ClO₂) residual (mg/L = ppm) in dump tank and flume water across all facilities and dates of survey assessment. Under the operational conditions, no functional correlation was observed.

Table 3. Comparison of total aerobic mesophiles (A) and total coliforms (B) populations between dump tank and flume system.

A.

		Total aerobic mesophiles (CFU/mL)						
		Dump tank			Flume system			
Facility	Trial	Mean	Std. dev.	Median	Mean	Std. dev.	Median	D log flume-dump
A	1	3.99	0.38	4.09	3.79	0.64	3.80	-0.20
	2	3.45	0.97	3.32	3.62	0.90	3.64	0.17
B	1	3.78	0.56	3.93	2.97	0.97	2.84	-0.81
	2	3.53	0.88	3.36	3.05	0.72	3.02	-0.49
C	1	4.74	1.12	4.59	2.30	0.64	2.19	-2.43
	2	4.71	1.22	4.93	1.12	0.63	0.99	-3.59
	3	4.63	1.19	4.84	2.92	2.37	1.00	-1.70

B.

		Total coliforms (CFU/100 mL)						
		Dump tank			Flume system			
Facility	Trial	Mean	Std. dev.	Median	Mean	Std. dev.	Median	D log flume-dump
A	1	4.79	1.27	4.84	3.68	1.87	4.09	-1.10
	2	3.95	1.82	4.20	3.72	1.48	4.21	-0.24
B	1	5.02	0.67	4.87	3.29	2.14	4.16	-1.73
	2	4.20	2.44	5.47	2.21	1.92	0.99	-1.99
C	1	3.15	1.68	3.06	1.12	0.47	0.99	-2.02
	2	5.07	1.37	5.31	1.11	0.48	0.99	-3.96
	3	2.84	1.02	3.38	1.66	1.07	0.99	-1.17
	4	5.06	0.73	5.38	2.78	1.03	2.65	-2.28

Table 4. Comparison of bacterial population of total aerobic mesophiles (A) and total coliforms (B) between incoming tomatoes in the dump tank and tomatoes exiting the flume and spray-wash system.

A.

		Total aerobic mesophiles (log CFU/fruit)						
		Before Washing			After washing			
Facility	Trial	Mean	Std. dev	Median	Mean	Std. dev	Median	Δ log change
A	1	4.98	1.03	4.53	4.67	0.70	4.43	-0.31
	2	4.06	1.06	4.40	4.39	0.32	4.46	0.33
B	1	4.62	0.66	4.40	4.39	0.69	4.36	-0.23
	2	4.86	0.55	4.67	4.60	0.86	4.45	-0.26
C*	1	3.19	0.74	3.19	4.16	0.46	4.22	0.96
	2	3.26	0.84	3.36	4.13	0.56	4.19	0.88
	3	4.54	0.97	4.58	3.57	0.98	3.63	-0.97

B.

		Total coliforms (log CFU/fruit)						
		Before Washing			After washing			
Facility	Trial	Mean	Std. dev	Median	Mean	Std. dev	Median	Δ log change
A	1	3.78	1.11	3.90	3.35	0.80	3.41	-0.43
	2	3.19	1.12	3.53	3.41	0.62	3.45	0.22
B	1	3.12	1.23	3.32	2.91	0.54	3.09	-0.21
	2	3.02	1.34	3.69	3.39	1.01	3.63	0.37
C*	1	1.44	0.78	0.99	2.91	0.74	2.97	1.47
	2	2.00	1.10	2.01	4.13	0.56	4.19	2.13
	3	2.51	1.24	2.38	2.65	1.00	2.71	0.14

*For trial number 4 these parameters were not determined.

Table 5. Mean pulp temperature of incoming and outgoing tomato fruit

Facility	Trial	Water Temperature (°C)		Tomato Temperature (°C)			Air Temp.
		Dump	Flume	Top	Bottom	After Washing	
A	1	34.6	35.9	24.5	26.2	30.3	
	2	35.1	35.5	29.7	29.2	31.4	
B	1	36.8	37.0	27.3	26.8	27.6	
	2	37.4	37.5	28.6	28.3	26.1	
C	1	34.2	43.0	28.6	24.1	25.8	28.5
	2	34.4	42.7	26.6	22.7	22.5	26.7
	3	33.6	42.1	21.6	21.4	24.2	23.6
	4	35.1	44.1	ND	ND	ND	ND

ND – Not done on this date as fruit were not collected for microbiological analysis.

Synthetic water experiments

Effect on ORP

- ✓ An inverse correlation was determined between water turbidity, temperature, and ORP.
 - Increase in water turbidity reduce the final ORP reached in the system, however values of ORP greater than 600 mV were reached in a time period of 5 seconds except when water turbidity is greater than 160 FAU, regardless of the single pulse chlorine dioxide dose added (Fig. 5-6).
 - Residual chlorine dioxide was reduced after 5 sec of initial addition, however lower loses of chlorine dioxide were detected at lower temperatures which also corresponded with higher ORP values (Tables 9-11).
- ✓ A direct relation was determined between water turbidity and temperature with pH (Fig. 7-8)

Effect on *Salmonella enterica*

Three doses of ClO₂ were utilized; 1, 3 and 5 mg/L. At a starting concentration of 10⁷ CFU/mL *S. enterica* sv. Newport, at least a 6 log reduction was observed for all concentrations, however the contact time for total inactivation of this bacterium was influenced by physicochemical parameters in the synthetic water (Tables 6-8).

- ✓ Increase in water turbidity increases the contact time for required inactivation of *S. enterica* at any water temperature
- ✓ Increase in temperature and dose of chlorine dioxide reduced the contact time to achieve a 6-log reduction (Table 6).
- ✓ Results were compared with the effect of several doses of NaOCl (5, 25 and 50 mg/L) [Table 7]. With exception of the lowest dose of NaOCl using water adjusted to 160 FAU, the contact time for inactivation of *S. enterica* was less than for ClO₂. However the log reduction within a 2 min timeframe was similar to that obtained with ClO₂.
- ✓ Additionally, substantial differences in contact time were observed for different *S. enterica* serovars (Table 8).

CONCLUSION AND RECOMMENDATION

The data-based delineation of industry challenges and a descriptive characterization of critical operating standards for chlorine dioxide in disinfection of dump tank and flume water for fresh tomatoes were accomplished. As an alternative to the use of chlorine and hypochlorite-based treatments, ClO₂ remains a viable option for some unit operations but significant obstacles for dump tank management were revealed. The adoption of industry-wide audit criteria should be carefully structured around these plausible limitations so as not to unnecessarily destroy tomatoes or potentially place the consuming public at risk.

Table 6. Effect of chlorine dioxide concentration, water turbidity and water temperature on inactivation time of *Salmonella enterica* sv. Newport.

	1mg/L ClO ₂			3mg/L ClO ₂			5mg/L ClO ₂		
	Temperature (°C)			Temperature (°C)			Temperature (°C)		
	10	25	40	10	25	40	10	25	40
Water turbidity (FAU)	Contact time needed for inactivation (sec) ¹								
Water	*	60	5	*	10	<5	*	10	<5
22	45	90	45	>120	15	5	>120	5	<5
43	>120	>120	90	>120	30	5	>120	5	<5
160	>120	>120	>120	>120	>120	>120	>120	90-120	75

¹ Initial concentration of *S. enterica* culture was 7 log CFU/mL

(*) Not determined for this condition

Table 7. Effect of sodium hypochlorite concentration and water turbidity on inactivation time of *Salmonella enterica* sv. Newport at 25°C.

NaOCl concentration (mg/L)	5	25	50
Water turbidity (FAU)	Time needed for inactivation (sec) ¹		
Water	5	<5	<5
43	120	15	<5
160	>120	75	15

¹ Initial concentration of *S. enterica* culture was 7 log CFU/mL

Table 8. Comparison of the effect of the concentration of Chlorine dioxide and water turbidity on different *Salmonella enterica* strains.

<i>S. enterica</i> strain	Newport (PTVS 077)	Newport + stress (PTVS 082)	Gaminara (PTVS 041)	Poona (PTVS 026)	Enteritidis (PTVS 044)	Agona (PTVS 043)	Montevideo (PTVS 045)	Michigan (PTVS 042)
1 mg/L of ClO₂								
Water turbidity (FAU)	Contact time (sec) needed for inactivation ¹							
43	>120	>120	>120	>120	>120	>120	>120	>120
160	>120	>120	>120	>120	>120	>120	>120	>120
3 mg/L of ClO₂								
Water turbidity (FAU)	Contact time (sec) needed for inactivation ¹							
43	30	30	60	75	120	>120	>120	120
160	>120	>120	>120	>120	>120	>120	>120	>120
5 mg/L of ClO₂								
Water turbidity (FAU)	Contact time (sec) needed for inactivation ¹							
43	5	5	30	60	90	90	120	120
160	90-120	120	>120	>120	>120	>120	>120	>120

¹ Initial concentration of *S. enterica* culture was 7 log CFU/mL

Figure 5. Example of the effect of water turbidity on oxidation reduction potential.

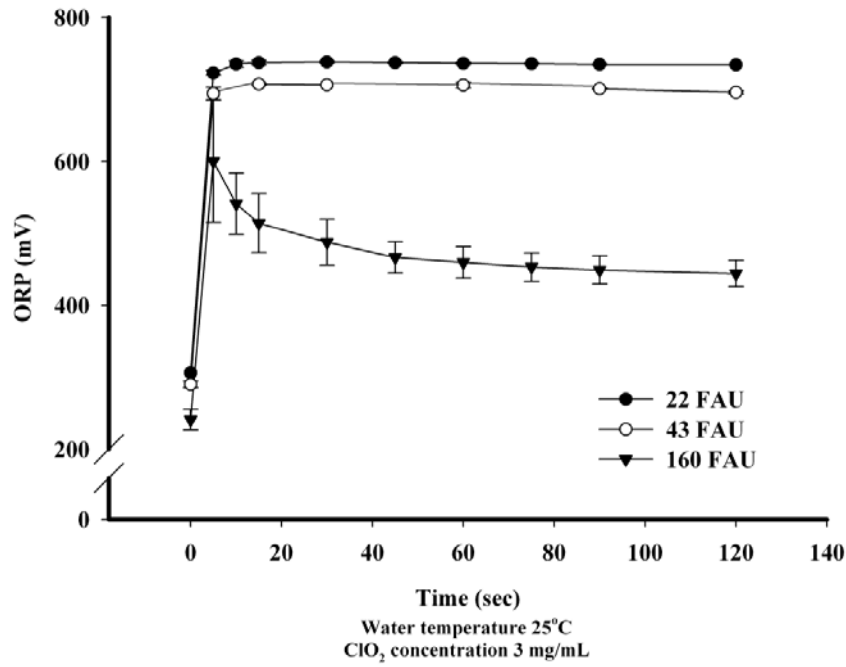


Figure 6. Example of the effect of water temperature on oxidation reduction potential.

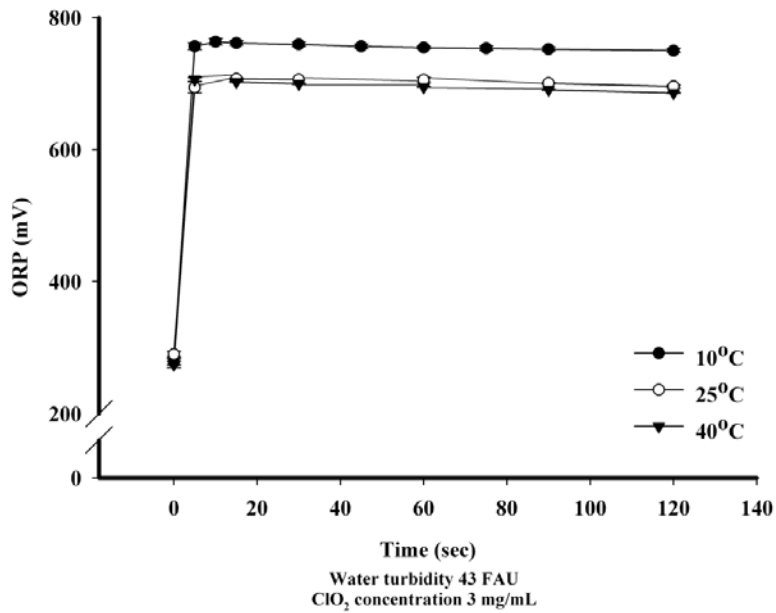


Figure 7. Example of effect of water turbidity on pH

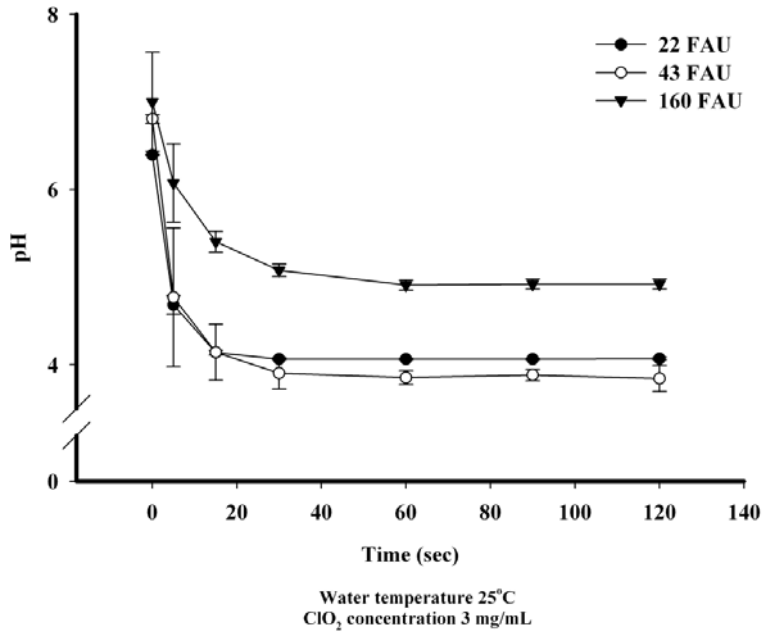


Figure 8. Example of effect of water temperature on pH

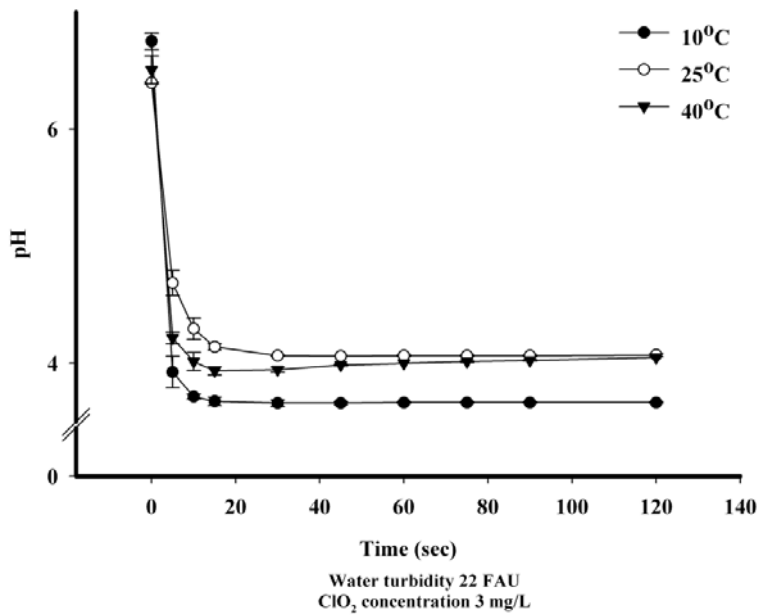


Table 9. Effect of water turbidity, water temperature and chlorine dioxide concentration on oxidation reduction potential (ORP).

Temperature (°C)	ORP (mV)								
	10			25			40		
	1	3	5	1	3	5	1	3	5
Time (sec)	22 FAU								
0	294	294	294	290	306	279	302	289	280
5	615	745	759	638	723	742	657	728	741
30	693	759	765	674	738	753	698	732	749
120	694	759	766	666	734	749	680	722	739
Time (sec)	43 FAU								
0	279	279	279	292	290	297	270	275	283
5	645	757	772	601	694	717	571	706	724
30	682	760	769	605	706	729	533	700	721
120	666	750	760	550	696	717	392	686	707
Time (sec)	160 FAU								
0	252	272	281	249	241	273	216	219	225
5	271	266	538	275	600	686	233	528	473
30	283	459	686	281	488	652	274	427	634
120	290	426	659	280	444	549	282	388	529

Table 10. Effect of water turbidity, water temperature and chlorine dioxide concentration on pH.

Temperature (°C)	pH								
	10			25			40		
	1	3	5	1	3	5	1	3	5
Time (sec)	22 FAU								
0	6.8	6.8	6.5	6.8	6.4	6.5	6.5	6.5	6.5
5	5.6	3.9	4.5	5.8	4.7	4.3	5.4	4.2	4.2
30	5.0	3.7	4.4	5.1	4.1	3.8	4.7	3.9	3.9
120	4.4	3.7	3.8	4.7	4.1	3.8	4.6	4.1	3.9
Time (sec)	43 FAU								
0	6.8	6.7	4.6	6.9	6.8	6.5	6.6	6.5	6.6
5	6.3	5.1	4.0	6.0	4.8	4.4	5.7	4.5	4.0
30	6.2	4.1	3.7		3.9	3.7	5.3	4.0	3.8
120	5.9	3.9	3.7	4.9	3.8	3.8	4.9	4.0	3.8
Time (sec)	160 FAU								
0	6.6	6.3	6.8	6.9	7.0	6.8	7.0	7.0	7.1
5	6.5	305.1	5.9	7.0	6.1	4.9	6.9	5.8	6.2
30	5.9	5.5	4.8	6.7	5.1	4.3	6.5	5.3	4.5
120	5.8	4.9	4.2	6.5	4.9	4.4	6.0	5.2	4.3

Table 11. Effect of water turbidity, water temperature on the residual of chlorine dioxide concentration during time.

Residual of ClO₂ (mg/L)									
Temperature (°C)	10			25			40		
[ClO ₂] mg/L	1	3	5	1	3	5	1	3	5
Time (sec)	22 FAU								
5	0.67	3.30	4.61	0.66	2.57	3.99	0.55	2.24	3.54
15	0.69	3.14	4.39	0.64	2.45	3.77	0.50	109.12	3.40
30	0.69	2.96	4.49	0.67	2.38	3.83	0.57	2.15	3.39
60	0.73	2.91	4.32	0.60	2.25	3.62	0.51	1.91	3.31
120	0.67	3.04	4.20	0.57	2.21	3.61	0.48	1.97	3.08
Time (sec)	43 FAU								
5	0.37	2.87	4.05	1.01	3.19	3.94	1.06	3.06	4.43
15	0.28	2.11	3.87	1.05	3.24	3.90	1.03	3.04	4.65
60	0.20	1.96	3.62	1.00	3.00	3.77	0.95	2.79	3.97
120	0.19	2.01	3.66	0.94	2.85	3.40	0.88	2.28	3.33
Time (sec)	160 FAU								
5	0.49	1.92	1.58	0.43	1.13	1.93	0.43	1.35	0.26
30	0.50	1.16	1.76	0.46	0.99	1.50	0.36	0.97	0.21
120	0.50	1.17	1.59	0.32	0.96	1.42	0.39	0.84	0.23