ICA TriNova, LLC
Intellectual Capital Associates

1 BEAVERS STREET - SUITE B - NEWNAN, GEORGIA 30263 - PHONE 770.683.9181 - FAX 770.251.7094

30 November 2015

Program Manager, USDA/AMS/TM/NOP
Room 4008-So., Ag Stop
1400 Independence Avenue, SW
Washington, DC 20250

Attn: Ms. Jessica Walden

Dear Jessica:

ICA TriNova LLC encourages the listing of Chlorine Dioxide (ClO_2) gas on the National Organics Program's list of "Allowed Substances". As detailed in the attached petition ClO_2 gas treats produce items directly. This approach provides superior efficacy against spoilage organisms and pathogens. ClO_2 gas is not a derivative of nor should it be confused with gaseous chlorine (Cl_2). Application of ClO_2 gas on fruits and vegetables produces no deleterious by-products. There is no residual to rinse or de-chlorinate and there is no potential to form unwanted chlorinated organics. It is also important to note that ClO_2 gas has been reviewed and received EPA and FDA approvals for uses on produce items.

Market suppliers of fresh and processed fruits and vegetables have a need for new interventions as they work to conform to new food safety rules and reduce supply chain loses. There is a large and growing body of science showing gaseous ClO_2 treatments are superior to traditional water rinse, spray or fogging treatments for improving produce quality and safety. Liquid ClO_2 generated by reacting sodium chlorite solution with organic or mineral acids is already recognized as organic, and ClO_2 gas is demonstrably better. We believe granting "Organic" status for ClO_2 gas will greatly facilitate market acceptance of new product interventions that will help growers, distributors, retailers and consumers with safety and sustainability goals.

We appreciate your consideration regarding this matter.

Sincerely,

Steven C. Beers
Partner, ICA TriNova, LLC
770.883.6410
stevenbeers@icatrinova.com

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Petition for Addition of a Synthetic Substance for Inclusion on the National List

Date: 30 November 2015

Name of Petitioner: ICA TriNova, LLC (Manufacturer and Distributor)
1 Beavers Street
Newnan, Georgia 30263
Phone: 770.683.9181
sales@icatrinova.com

Contact: Steven C. Beers, Partner
Phone: 770.883.6410
stevenbeers@icatrinova.com

Item A: Sections for Inclusion – Section § 205.605(b)
Synthetic or non-synthetic nonagricultural (non-organic) substances allowed in or on processed products labeled as “organic” or “made with organic (specified ingredients)”.

Item B: Background Information Regarding Substance Being Petitioned

1. Substance Chemical Name – Chlorine Dioxide (ClO_2) Gas
   Substance Material Common Name – Chlorine Dioxide (ClO_2) Gas
2. Manufacturer/Producer’s Name and Contact Information
   a. ICA TriNova, LLC (Manufacturer and Distributor)
      1 Beavers Street
      Newnan, Georgia 30263
      Phone: 770.683.9181
      sales@icatrinova.com
   b. Steven C. Beers, Partner
      Phone: 770.883.6410
      stevenbeers@icatrinova.com
3. Current Use – Z-Series™ FruitGard® (chlorine dioxide in gas form) is a registered anti-microbial pesticide, sanitizer and/or disinfectant for fruits and vegetables (FCN 949, EPA Reg. # 79814-5) used for the direct treatment of fruits and vegetables during storage, transportation and food preparation applications with no requirement for post treatment rinse.
4. Substance Usage - The substance is used in the direct treatment of vegetables, fruits, nuts, to reduce spoilage and pathogenic organisms. In these applications the mode of chlorine dioxide is as a killing agent of these organisms. The substance is applied as a dry pure gas in closed containment. Treatments are done over several hours such that the substance is completely consumed or nearly so. Application rates will vary

by article type. However, in all cases it has been demonstrated that chlorine dioxide will be converted to chloride ion on the articles.

5. Source of Substance and Manufacturing Process.
    a. Materials for listed product are obtained from two sources
        i. Zeolite substrate: Zeotech Corporation, Fort Worth, Texas
        ii. Chemicals: All chemicals utilized in the production process (as listed in **Exhibit 5a**) are purchased from Industrial Chemicals Corporation, Birmingham, Alabama.

    b. Manufacturing Process.
        See **Exhibit 5b**

6. Summary of Previous Reviews by State or private certification programs or other organizations of the petitioned substance.
    a. **Exhibit 6b,** USDA Published Paper: "Distribution and Chemical Fate of ^{36}Cl-Chlorine Dioxide Gas during the Fumigation of Tomatoes and Cantaloupe", *Journal of Agricultural and Food Chemistry*, D.J. Smith, *et al*, November 2014, pp 11756-11766.

    b. **Exhibit 6c,** USDA Published Paper: "Chloroxyanion Residues in Cantaloupe and Tomatoes after Chlorine Dioxide Gas Sanitation", *Journal of Agricultural and Food Chemistry*, D.J. Smith, *et al*, October 2015, pp 9640-9649.

    c. USDA, ARS, NPA, NRRC Trust Fund Cooperative Agreement – "Magnitude and Nature of Chemical Residues Present on Produce and Meats after Treatment with Chlorine Dioxide Gas". Agreement number 58-5442-1-431.

7. Information regarding EPA, FDA and State Regulatory Authority Registrations.
    a. USEPA Reg # 79814-5, FruitGard®
    b. Various State Pesticide Registrations – FruitGard®
    c. FDA FCN 949 – ClO_2 Gas Treatment for Processed Fruits and Vegetables

8. Chemical Abstract Service (CAS) Number and Labels
    a. CAS – none
    b. Substance(s) Commercial Labels and instruction Sheets
    Exhibit 8b.1 – FruitGard® label and instruction sheet

9. Substance Physical Properties and Mode of Action –
    a. Chemical Interactions - Chlorine dioxide gas is a known oxidizer; however, in the prescribed applications described herein, there are no known interactions with other substances used in organic production.

    b. Toxicity and Environmental Persistence - Chlorine dioxide is not persistent nor a known bio-accumulative substance –

(ref: EPA/635/R-00-007, Toxicological Review of Chlorine Dioxide and Chlorite, Sept 2000)

c. Environmental Impacts from Use and/or Manufacture - There are no known environmental impacts from the uses patterns described herein.

d. Effects on Human Health - The toxicity of chlorine dioxide is well established; it is not a known carcinogen or mutagenic substance – (ref: EPA/635/R-00-007, Toxicological Review of Chlorine Dioxide and Chlorite, Sept 2000). The primary concern of exposure to the substance is acute toxicity related to inhalation where the substance is a known irritant to eyes and mucal membranes. Severe exposure (beyond amounts available by petitioned product) can result chemically induced pneumonia and or death.

e. Effects on Soil Organisms, Crops, or Livestock
   i. Chlorine dioxide has been shown to decrease soil organisms when applied in concentrated form as liquid or a gas. The literature shows how bacteria populations can be suppressed, and larger insects, *e.g.* nematodes, can be reduced in a variety of soil types, *e.g*. potting soils.
   ii. Chlorine dioxide has been shown to be phytotoxic to a variety of plants when applied in concentrated form as liquid or gas. However, the literature shows how low gas concentrations can be used to control a number of root and foliage diseases and when used as a water disinfectant plants are known to be more robust to exposure than to alternative disinfectants, *e.g.* chlorine.
   iii. Concerns regarding livestock exposures to chlorine dioxide gas will largely parallel those of humans. However, chlorine dioxide has been shown to be safe in many applications and has widespread use as a water disinfectant and cleaning agent within the animal health industry.
   iv. The discussion above largely relates to concentrated chlorine dioxide use patterns. The substance related to this petition is applied at low levels and in secure conditions such that these concerns are greatly mitigated or not relevant.

10. Safety Data Sheets (SDS – formerly MSDS); National Institute of Health Report
    a. National Institute of Health Report – None known
    b. **Exhibit 10** - ClO_2 Dry Gas SDS' (FruitGard®)

11. Substance Research Information
    See **Exhibit 11**.

12. Petition Justification Statement
    a. Inclusion of a Synthetic on the National List, § 205.605(b)
       The substance has been demonstrated to produce no toxic or harmful residues when used in treating produce of all kinds.

b. Explain why the petitioned substance is necessary for the production of handling of an organic product.

The substance will improve the shelf life and safety of treated articles. It represents a significant new tool to advance current food safety directives and needs.

c. Describe any substances on the National List or alternative cultural methods that could be used in place of the petitioned substance.

There are no known gas substance equivalents. However, chlorine dioxide as applied in liquid form is listed.

d. Describe the beneficial effects to the environment, human health, or farm ecosystems from use of the petitioned substance that support its use instead of the use of any other substance or alternative cultural method.

With respect to societal and environmental benefits, chlorine dioxide has superior qualities to chlorine the most widely used intervention:

1- ClO_2 nor its precursors carry the EPA and Homeland Security reporting requirements attendant to chlorine gas.
2- ClO_2 does not create harmful by-product contamination *e.g*. Trihalomethanes (THMs) and Haloacetic acids (HAAs).
3- ClO_2 reacts rapidly and completely thereby reducing or negating the need for de-chlorination of waste water streams.
4- ClO_2 is effective over a broad pH range. Unlike chlorine it does not dissociate in water whereby the concentration it's most effective form HOCl can vary with water pH.

Chlorine dioxide applied in liquid form is already recognized as an organic substance. The substance petitioned by this submission is pure (dry) chlorine dioxide gas which has benefits beyond traditional liquid applications. Because gaseous chlorine dioxide treats target items directly significant additional advantages are achieved:

1- Liquid applications mainly treat rinse waters and do little to reduce organisms on the produce (< 2 log reductions); gas effectively treats the produce surfaces and thus provides superior efficacy (> 3 log reductions).
2- Gas applications often result in less chemical being used.
3- The dry application of gas results in better penetration of coarse or porous produce surfaces; yielding efficacy on hard to treat stem scars and wounds.
4- USDA research has demonstrated chlorine dioxide gas has different by-product distributions than chlorine or chlorine dioxide liquid. Gas produces no chlorinated organics and favors the full oxidation reaction of chlorine dioxide; as such chloride ion is the most favored and predominant measured

by-product on produce surfaces. There is no potential for residual chlorine dioxide or chlorite ion.

5- Gas phase chlorine dioxide interventions are superior to traditional water rinse, spray or fogging treatments for improving shelf life and food safety of produce.

6- Gas chlorine dioxide has numerous potential application points in the value chain. Emerging technology like FruitGard® ClO_2 provide novel, safe and easy methods to apply gas from the farm to the fork.

7- In some intervention concepts, gas treatments have the potential to supplant more commonly used sanitizing wash treatments, thereby reducing water consumption and eliminating the need for post treatment de-chlorination of treatment streams before discharge.

8- With its flexibility and ease of use for treating produce piles (*e.g.* grain silos and potato stocks), gas phase ClO_2 has highly impressive implications for industry and societal sustainability goals to reduce the dramatic post-harvest rot (moisture engendered) losses of produce and grains in storage inventories.

13. Confidential Business Information – None

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Item B
Statement #5
Exhibit 5
Manufacturing Processes

ClO_2 DRY GAS MEDIA

FruitGard®

ICA TriNova, LLC
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Manufacturing Process – ClO_2 DRY GAS MEDIA

(ZC) 3.0% - 400 lb. load (FruitGard® and CoilCleaner™)

MIXER # _____

Sodium Chlorite Impregnate

Batch Production Record – Inventory Qty. = 223,200 grams

Materials:

(6x14T (clean) Zeolite)	Material 1
(Chlorite Solution 25 wt % (31.25 wt %))	Material 2
(Selected Buffer, if any, ____ wt % Sol)	Material 3

Procedure:

ZEOLITE Confirm Correct Zeolite Size _____ PPE- SAFETY GOGGLES, DUST MASK, STEEL TOE BOOTS, GLOVES, EAR PROTECTION,	**CHEMICALS** **Confirm Chemicals (Name, Lot # amount) _____** PPE: GOGGLES, GLOVES, PROTECTIVE CLOTHING
Make sure mixer is in "locked" or "tow" position. Pour zeolite into mixer and cover mixer. CONFIRM CORRECT AMOUNT OF ZEOLITE.	Dispense chemicals into drum. CONFIRM CHEMICALS INJECTED (Name, Lot #, amount) Gently mix chemicals with stir bar.

- ◯ Turn on mixer and engage blades.
- ◯ Confirm that spray heads are properly positioned. Turn on sprayer.
- ◯ When all chemicals have been applied to the zeolite, (1)turn off sprayer, (2)disengage mixer blades, (3) Turn off mixer.
- ◯ Empty the mixer into drums – put media into proper receptacle.
- ◯ Keep media covered when not in use.

TAP DENSITY TEST PROCEDURE: Weigh out 100 grams ZC. Put ZC into plastic graduated cylinder. Tap cylinder firmly on its base 25 times. Record level of ZC on chart below.

TAP DENSITY TEST 100 GMS	
INITIALS	**Cylinder Reading**

TAP DENSITY TEST 100 GMS	
INITIALS	**Cylinder Reading**

TAP DENSITY TEST 100 GMS	
INITIALS	**Cylinder Reading**

SODIUM CHLORITE
LOT #

OTHER
NAME

LOT#

Production Lot Number:	**No. Batches Produced:**
ZC3.0- -006	
PRODUCTION MANAGER:	

Place FINAL FruitGard Lot# Label Here:

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Item B
Statement #6
Exhibit 6b&6c

Substance Review Documents

1. “Distribution and Chemical Fate of ^{36}Cl-Chlorine Dioxide Gas during the Fumigation of Tomatoes and Cantaloupe”

2. “Chloroxyanion Residues in Cantaloupe and Tomatoes after Chlorine Dioxide Gas Sanitation”

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Distribution and Chemical Fate of ^{36}Cl-Chlorine Dioxide Gas during the Fumigation of Tomatoes and Cantaloupe

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ABSTRACT: The distribution and chemical fate of ^{36}Cl-ClO_2 gas subsequent to fumigation of tomatoes or cantaloupe was investigated as were major factors that affect the formation of chloroxyanion byproducts. Approximately 22% of the generated ^{36}Cl-ClO_2 was present on fumigated tomatoes after a 2 h exposure to approximately 5 mg of ^{36}Cl-ClO_2. A water rinse removed 14% of the radiochlorine while tomato homogenate contained ∼63% of the tomato radioactivity; 24% of the radiochlorine was present in the tomato stem scar area. Radioactivity in tomato homogenate consisted of ^{36}Cl-chloride (≥80%), ^{36}Cl-chlorate (5 to 19%), and perchlorate (0.5 to 1.4%). In cantaloupe, 55% of the generated ^{36}Cl-ClO_2 was present on melons fumigated with 100 mg of ^{36}Cl-ClO_2 for a 2 h period. Edible cantaloupe flesh contained no detectable radioactive residue (LOQ = 0.3 to 0.4 μg/g); >99.9% of radioactivity associated with cantaloupe was on the inedible rind, with <0.1% associated with the seed bed. Rind radioactivity was present as ^{36}Cl-chloride (∼86%), chlorate (∼13%), and perchlorate (∼0.6%). Absent from tomatoes and cantaloupe were ^{36}Cl-chlorite residues. Follow-up studies have shown that chlorate and perchlorate formation can be completely eliminated by protecting fumigation chambers from light sources.

KEYWORDS: *cantaloupe, chlorate, chlorine dioxide, chloroxyanion, food safety, fumigation, perchlorate, tomato*

INTRODUCTION

The Food and Agriculture Organization of the United Nations estimated in 2011 that approximately 1.3 billion tons of foods (1.3×10^{12} kg) are lost annually through spoilage or waste[1] across all levels of the production, transport, retail, and consumer cycle. Lost and wasted food is estimated to represent one-third of annual global food production. As the world population increases, demands for greater efficiencies of land, water, and energy use for food production will escalate. In industrialized countries, intensive efforts in crop breeding, agronomic practices (i.e., use of fertilizers, modifications of tillage technique, and use of herbicides and pesticides), and modification of plants through molecular biology (i.e., generation of herbicide resistant commodity crops) have largely met increased efficiency demands. Future enhancements will, by necessity, focus on harvesting efficiencies, product distribution, and increases in shelf-lives for products prone to spoilage. However, an assumption implicit with technological improvements in perishable food distribution and preservation is that improvements must occur without compromising the safety of consumers.

For pomes, vegetables, berries, melons, leafy vegetables, and most other crop groups[2] there are a variety of spoilage organisms[3] that can quickly and irreversibly reduce quality during the interval from harvest to market. Spot spoilage limits the acceptability of otherwise healthy products in developed countries and severely limits distribution of food products in developing countries. In addition, microbial colonization of vegetable foods increases risks associated with nonrot organisms. For example, mycotoxins[4] and specific human pathogens including, but not limited to, *Clostridium botulinum*, *Listeria monocytogenes*, *Salmonella*, shigella-toxin producing *Escherichia coli*, *Cryptosporidium*, *Cyclospora*, and a number of viruses[5] are commonly associated with vegetable food products. Collectively, food spoilage organisms, human pathogens, and mycotoxin producing organisms represent huge, but preventable, losses to global food production systems. In recognition of these losses and their implications for human suffering, intensive scientific efforts at improving the storage, transport, safety, microbial cleanliness, and distribution of perishable food items have been undertaken.

One technology that has resulted from this effort is the use of chlorine dioxide as a disinfectant and sanitizing agent. As early as 1967, aqueous chlorine dioxide rinse solutions were approved for applications as diverse as fruit, vegetable, and meat washes, odor control, and food equipment disinfection.[6] In 1988, chlorine dioxide was approved as a sterilant for laboratory surfaces, for environmental surfaces, for tools, and for clean rooms.[6] Gaseous chlorine dioxide, however, is also an effective fumigant for the reduction or elimination of rot and (or) pathogenic microbial species on a variety of crop groups,[7−11] and its applications for preventing food spoilage and contamination are obvious. To date, however, chlorine dioxide gas for the treatment of human vegetable foods has not been approved for use in the United States because chemical residues in food matrices after chlorine dioxide gas application have not been definitively characterized.

The purpose of this study was to investigate the fate, distribution, and transformation of radiolabeled chlorine

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dioxide in fumigated tomatoes and cantaloupe. An additional objective was to investigate the effect of laboratory illumination on the formation of chloroxyanion byproducts such as chlorate and perchlorate during chlorine dioxide fumigation.

MATERIALS AND METHODS

Radiolabel. Stock $Na^{36}ClO_2$, having a radiochemical purity of 90.5% and a specific activity of 14,000 dpm/μg was generated from $Na^{36}ClO_3$ as described by Hakk et al.[12] The 9.5% radiochemical impurity present in the stock $Na^{36}ClO_2$ solution was $Na^{36}Cl$ as determined by ion chromatography with radiochemical detection. Radioactive chlorine dioxide ($^{36}ClO_2$) was generated by the mineral acid catalyzed oxidation of $Na^{36}ClO_2$ (aq). For the tomato studies, 315 μL of stock $Na^{36}ClO_2$ was combined with 36 μL of sodium chlorite technical solution (318 mg/mL by iodometric titration; ICA TriNova), to a specific activity of 389 dpm/μg, and 49 μL of water in a Tyvek sachet (2.6 × 13 cm). Acidification of the chlorite solution with 1.8 M HCl (250 μL) initiated the release of $^{36}ClO_2$. The specific activity of the $^{36}ClO_2$ gas was 521 dpm/μg.

For the cantaloupe studies, sequential volumes of 4.925 mL of water; 1.390 mL of stock $Na^{36}ClO_2$, corrected for radiochemical purity; 0.628 mL of sodium chlorite technical solution; and 4.340 mL of 1.8 M HCl were added to a Tyvek sachet (19 × 5 cm, L × W) to initiate $^{36}ClO_2$ release. The specific activity of the $^{36}ClO_2$ gas was 134 dpm/μg.

Tomato Studies. *Tomato Fumigation.* Three separate $^{36}ClO_2$-fumigation experiments (trials A, B, and C) were conducted. In each experiment, approximately 100 g of tomato was exposed to approximately 5 mg of $^{36}ClO_2$ gas during a 2 h fumigation period. Fumigations occurred within a 5.5 L (11 × 22 × 23.5 cm; W × L × D) sealable glass tank (Figure 1). During experiments A and B, no effort was made to protect the fumigation tank from light, but for trial C the fumigation tank and lid were each protected from laboratory illumination by an aluminum foil wrap. Fumigation tanks were placed onto a magnetic stirring plate, and tomatoes were placed onto a slotted glass pedestal within each tank. A stir bar was also placed in the glass chamber and was allowed to rotate during fumigation to facilitate mixing of gases. Reactions were initiated, and the reaction chamber was sealed with a glass plate previously lined with vacuum grease. Glass lids were equipped with butyl-stoppered (20 mm; Kimble Chase; Vineland, NJ) entry and exit holes through which gases could be purged. Exposure periods were 2 h each. Experimental protocols varied slightly between experiments A, B, and C, and these variations are shown in Table 1.

Recovery of Chamber Gas Radioactivity. At the end of each fumigation experiment unreacted gas was either released into the fume hood (trial A) or trapped into 2 L of 0.1 N sodium thiosulfate after air was pumped (0.6 to 1.0 h) through the exposure chamber (trials B and C). To this end, entry and exit septa of the chamber lids were pierced with 11-gauge needles connected to Tygon tubing; gas pumped through the fumigation chambers was passed through coarse micro gas dispersion tubes (Chemglass Life Sciences, Vineland, NJ) housed within the thiosulfate.

Recovery of Tomato Radioactivity. Tomatoes were removed from the reaction vessel using tongs and placed into a 250 mL beaker containing about 200 mL of water. Tomatoes were rinsed for about 1 min, after which the tomato was removed and the rinsewater placed into a 250 mL volumetric flask; the beaker was rinsed, and the rinsewater was added to the volumetric flask. The volumetric flask was diluted to the mark and mixed, and radiochlorine in 1−2 mL aliquots was quantified by liquid scintillation counting (LSC).

Recovery of Chamber Residual Radioactivity. Stir bars and glass pedestals were placed in 250 mL beakers and rinsed with water, which was placed into volumetric flasks (0.5−1 L). Radioactive residues were recovered from the reaction chamber by rinsing sequentially with water. Rinses were transferred to the volumetric flask; the volumetric was diluted to the mark and mixed by inversion. Radioactivity in 1 to 2 mL aliquots was determined by LSC.

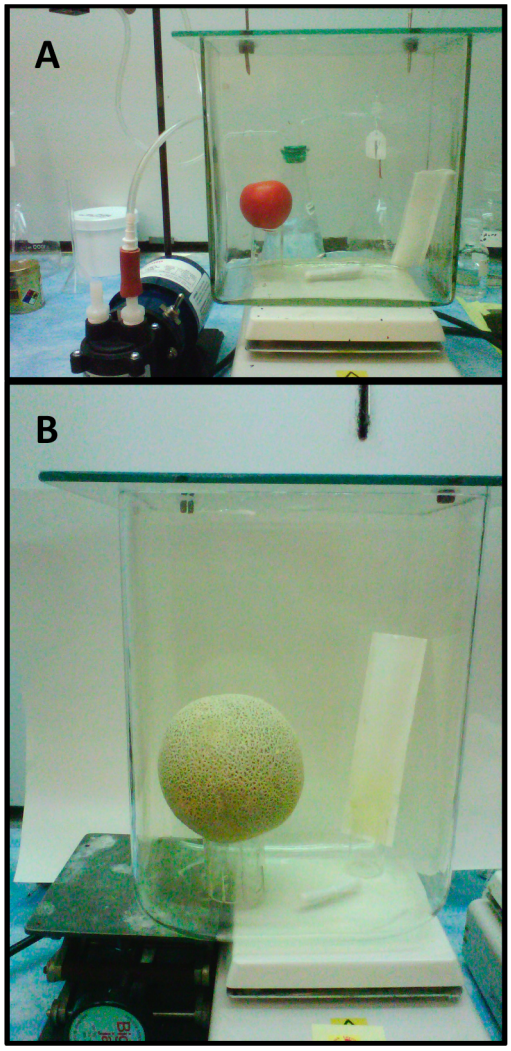

Figure 1. Experimental chambers for tomato (panel A) and cantaloupe (panel B) fumigations with ^{36}Cl-chlorine dioxide. Panel A shows a tomato experiment at the initiation of the venting process. Panel B shows a cantaloupe experiment during fumigation; ^{36}Cl-chlorine dioxide gas can be clearly seen as the greenish tint in the fumigation chamber. For each fumigation experiment, a slotted glass pedestal was used to support tomatoes or cantaloupe, a stir bar was used to provide gas circulation, and a Tyvek sachet contained the $^{36}ClO_2$ generating system. Glass lids were sealed with vacuum grease, and two butyl septa (embedded in the lids) served as portals through which air was pumped into sodium thiosulfate traps at the termination of the experiment. No effort was made to prevent laboratory illumination of either of the fumigations shown.

Radioactivity remaining within gas generation sachets was recovered after sequential rinsing with water and transfer of the rinse fractions into a 1 L volumetric as described for the tank rinse. Sachet radioactivity not removed by the water rinse was quantified after cutting each sachet into 1 cm strips and counting each strip directly in liquid scintillation fluid by LSC.

Recovery of Tomato Radioactivity. Rinsed tomatoes were weighed and homogenized whole (trial A), or the stem scar area was removed (trials B and C) and then the tomato was weighed and homogenized. The stem scar area was removed with a razor so that it contained minimal to no tomato skin or flesh. Aliquots (0.25 g) of puree were placed into glass LSC vials, digested overnight with 6 mL of Carbosorb E (PerkinElmer Life Sci.; Waltham, MA), and counted after the addition of 12 mL of Permaflour E (PerkinElmer Life Sci.; Waltham, MA) using LSC to obtain total radioactive residues (TRR). Stem scar

Table 1. Experimental Differences between Tomato Fumigation Trials A, B, and C

	variable		
trial	gas purge[a]	stem scar collection[b]	illumination[c]
A	no	no	yes
B	yes	yes	yes
C	yes	yes	no

[a]Unreacted chlorine dioxide gas was either purged and trapped from the reaction vessel or vented into the fume hood. [b]The stem scar was either left on the tomato during postexposure processing or removed and processed separately. [c]The reaction chamber was unprotected from light for the 2 h reaction period, or it was covered with aluminum foil during the fumigation period and protected from illumination.

areas were added to a known amount of water and (or) crushed ice and were homogenized in a 25 mL stainless steel Waring blender cup. Radioactivity in scar puree was then quantified by LSC as described for tomato puree.

Preparation of Tomato Serum and Pellet Fractions. Aliquots of tomato puree were fractionated into liquid serum and solid pellet fractions by centrifugation at 30600*g* for 20 min. Aliquots (0.5 mL) of the serum fraction were counted directly in 15 mL of Ultima Gold LSC fluid; TRR in pellet aliquots (0.25 g) was quantified by LSC after overnight treatment in 6 mL of Carbosorb E followed by dilution in 12 mL of Permafluor LSC fluid.

Speciation of Radioactive Residues in Tomato Rinse, in Serum Fractions, and in Tank Rinse Fractions. The strategy for identification and quantification of radioactive metabolites was to fortify aliquots of tomato serum or water rinse samples with nonradioactive chlorite, chloride, and chlorate (21 to 23 μg; for use as chromatographic markers) and to inject the fortified aliquots onto the ion chromatograph. Each metabolite fraction was collected into a LSC vial as it eluted from the detector, and radioactivity in each fraction was determined by LSC. This strategy is essentially the same as described by Smith et al.[13] for the analysis of radioactive chlorate metabolites in beef tissues. Chlorite, chloride, and chlorate were separated using a Dionex AS11-HC (Thermo-Fisher) column; for perchlorate analysis, a Dionex AS16-HC column was used (see conditions below). Guard columns were not used. For trial C, quantification of perchlorate in the tomato rinse fraction was accomplished using ion chromatography with conductivity detection (described below); conductivity detection was more sensitive than radiochemical determination and could be used because the rinse fraction was free of interferences.

Digestion of Tomato Pellets and Characterization of Radioactivity. Radioactivity associated with the pellet fraction formed during the centrifugation of the tomato puree was released by digestion of pellet aliquots (1 g) in 1 M NaOH (50 °C for 72 h). Subsequent to digestion, slurries were recentrifuged at 50000*g* and the supernatant was assayed to determine recovery of radioactivity. Aliquots of digesta supernatant were treated with 0.3 M silver nitrate to precipitate silver ^{36}Cl-chloride, as were aliquots of blank sodium hydroxide matrix, and sodium ^{36}Cl-chloride and sodium ^{36}Cl-chlorate fortified saline samples (controls). The amount of radioactivity remaining in supernatant aliquots was determined by LSC.

LSC Techniques. Background radiochlorine and limits of quantification were determined for individual matrices (i.e., tank rinse, tomato puree, tomato serum, tomato pellet, etc.) as described by Smith et al.[14] Individual aliquots of sample within a matrix set were counted for 10 to 20 min each. Radiochlorine was quantified using a Packard 1900 CA (Meriden, CT) liquid scintillation counter calibrated using a sealed radiochlorine standard (Analytics Inc., Atlanta, GA) prepared in Ultima Gold LSC fluid. Quench was corrected using the tSIE (transformed spectral index of the external standard; Packard) option. Net dpm of a sample was determined by subtracting the mean background dpm of a sample set from the gross dpm of a test sample.

Ion Chromatography. Chromatography was conducted using a Waters model 600 pump and controller having PEEK pump heads and tubing. For ^{36}Cl-chlorite, ^{36}Cl-chloride, and ^{36}Cl-chlorate analyses, 10 mM NaOH was isocratically pumped at 1 mL/min through a Dionex AS11-HC, 4 × 250 mm, column. A conductivity detector (Dionex CD-25; 0.1 V, 100 mA, range, 3000) with external water suppression (Dionex ASRS 300; 4 mm) was used to monitor the elution of sample components. ^{36}Cl-Perchlorate was separated using the same chromatograph equipped with a Dionex AS16-HC, 4 × 250 mm column with an isocratic mobile phase of 50 mM NaOH flowing at 1 mL/min. All samples were introduced through a Rheodyne 9725i Teflon injector equipped with a 1 mL injection loop. For each analysis, the detector signal was captured on paper using a Waters model 746 data module.

For some analyses of water rinses, perchlorate analysis was conducted using a Thermo-Fisher ICS-2100 ion chromatograph using the framework outlined in EPA method 314.0.[15] Briefly, sodium perchlorate standards (4, 10, 25, 100, 200, and 400 μg/L) were prepared in nanopure water. The limit of quantitation (LOQ) was equivalent to the lowest standard (4 μg/L) with the limit of detection (LOD) at 2 μg/L. Tomato rinse sample aliquots (1 mL) were injected onto the column, and perchlorate was separated from interferences on a Dionex AS16-HC column protected by an AG16-HC guard column (both 4 mm). An isocratic mobile phase of 50 mM KOH with a flow rate of 1.5 mL/min was generated using a Thermo-Fisher eluent generator. Perchlorate was measured using suppressed conductivity detection in-line with external water ASRS suppression (186 mA).

Fate of Sodium ^{36}Cl-Chlorite Injected into Tomatoes. Fifty individual grape tomatoes (average wt 6.6 g) were each washed with water, blotted dry, weighed, and injected with 50 μL of sodium $Na^{36}ClO_2$ (862 μg; 90.5% radiochemical purity; 99 dpm/μg of sodium chlorite; radiochemical impurity was $Na^{36}Cl$). The injected tomatoes were collectively transferred into a Cuisinart blender and homogenized as described above for fumigated tomatoes. Likewise, total radioactive residues and the composition of residues in injected-tomato serum was determined as described for fumigated tomatoes.

Cantaloupe Studies. *Cantaloupe Fumigation.* Two experiments were conducted; for each trial, a single 18-count (851.6 g, trial A; 850.0 g, trial B) cantaloupe was supplied by SunFed Produce, Nogales, AZ; or Frontera Produce, Honduras. Test $^{36}ClO_2$ was generated in a Tyvek sachet as described for tomatoes. After acid addition, the glass exposure chamber (23.2 × 17.1 × 32.3 cm; L × W × D; 12.9 L) was sealed as rapidly as possible. The glass lid used for sealing the chamber was equipped with two 1 cm ports which were each sealed with 2 cm butyl septa. Cantaloupes were treated for 2 h with $^{36}ClO_2$ gas to meet a target exposure of 100 mg of $^{36}ClO_2$ per kg of cantaloupe (Figure 1). Mixing of gases within the exposure chamber was accomplished with magnetic stirring. No attempt to protect either cantaloupe experiment from laboratory light was made. Average light intensity in the laboratory that the experiments were conducted in is 900 ± 17 lx.

Recovery of Chamber Radioactivity. Chamber gases were collected as described for tomatoes except that a 1 N sodium thiosulfate trapping solution was used. Exposure chambers were purged for 1 (trial A) or 1.5 (trial B) h each. Radioactive residues were recovered from glass surfaces of the reaction chamber and Tyvek sachets as described for tomatoes.

Recovery of Cantaloupe Radioactivity. Cantaloupe were not rinsed after fumigation. Each cantaloupe was bisected with a single stroke of a stainless steel blade, and the seed bed was carefully removed and weighed. Edible cantaloupe flesh was separated from the rind with careful attention directed toward not contaminating the edible flesh with dry or liquid material from the inedible rind. The edible cantaloupe flesh was placed into a clean container and weighed; the rind portion was sliced into manageable pieces and weighed. Seed bed, edible flesh, and rind fractions were homogenized (Cuisinart CB-500) separately, and the resulting purees were analyzed for TRR content by LSC as described for the tomatoes.

Preparation of Edible Flesh and Inedible Rind Serum and Pellet Fractions. Aliquots (50 mL) of edible flesh or inedible rind puree were fractionated into liquid (serum) and solid (pellet) fractions by centrifugation at 30600*g* for 20 min. The serum and pellet fractions were separated, and quintuplicate aliquots (0.5 to 1.0 mL) of the sera fractions were counted directly in 15 mL of Ultima Gold

Table 2. Distribution of Radioactivity after the Fumigation of Test Tomatoes with $^{36}ClO_2$ Gas

	trial A		trial B		trial C	
	wt (g)	act. (%)	wt (g)	act.[a] (%)	wt (g)	act. (%)
starting amounts[b]						
tomato wt	74.62		97.54		108.27	
$Na^{36}ClO_2$		90.5		90.5		90.5
$Na^{36}Cl$		9.5		9.5		9.5
total		100.0		100.0		100.0
tomato activity						
tomato rinse		1.9		2.2		1.1
puree	74.62	10.0	96.77	7.5	108.03	7.8
stem area puree	NA[c]	NA	0.64	3.9	0.24	2.2
tomato		11.9		13.7		11.0
gas purge		NM[d]		14.8		18.0
equipment rinse						
tank rinse		12.5		10.1		0.6
tank seal		NM		0.5		1.0
lid seal		NM		0.2		NM
equipment		12.5		10.8		1.6
nonsachet activity[e]		NC[f]		39.2		31.4
sachet activity						
sachet rinse		42.3		42.3		47.8
sachet		1.4		1.9		1.2
sachet		43.7		44.2		49.0
total recovery	74.62	*f*	97.41	83.5	108.27	80.4

[a]Expressed as a percentage of the starting radioactivity. [b]Total starting radioactivity was 2.27 μCi. [c]NA, the stem area was not removed in tomato trial A. [d]NM, not measured. [e]Nonsachet activity is the sum of total tomato activity, gas purge activity, and equipment rinse activity. [f]NC, not calculated because gas was not collected after the termination of the experiment.

(PerkinElmer; Waltham, MA) LSC fluid. Total radioactive residues in quintuplicate pellet aliquots (0.25 g for edible flesh, 0.05 g for inedible rind) were quantified by LSC after overnight treatment in 6 mL of Carbosorb E followed by dilution in 9 mL of Permafluor LSC fluid. Use of greater than 0.05 g of inedible rind pellet caused sample quench due to the intensity of color.

An untreated control cantaloupe was also fractioned into inedible rind, seed bed, and edible flesh fractions. In addition, control serum and pellet fractions were prepared from cantaloupe rind and edible flesh as described for treated melons. Sample aliquots from fractions of the control cantaloupe were used as blanks for determination of background radioactivity during the analysis of treated cantaloupe.

Speciation of Radioactive Residues. Radioactive residues in edible flesh, inedible rind sera, and tank rinse fractions were identified as described for tomatoes. Edible flesh serum from cantaloupe trial B, which contained TRR just above the detection limits, was thawed, and quintuplicate 1.25 mL aliquots of serum were fortified with 0.5 mL of 0.85% aqueous NaCl. Additional quintuplicate 1.25 mL aliquots of edible flesh serum were diluted with 0.5 mL of 0.3 M $AgNO_3$. Both sets of samples were centrifuged (15000*g* for 20 min), and 1.25 mL aliquots of each vial were transferred to glass LSC vials for determination of soluble radioactive residues in 15 mL of UltimaGold LSC fluid. For the saline treated serum, soluble radioactive residues would represent the TRR; for the $AgNO_3$ treated serum, the soluble residues would represent any residue present as ^{36}Cl-chlorite, ^{36}Cl-chlorate, and (or) ^{36}Cl-perchlorate.

Factors Impacting the Formation of Chloroxyanion Byproducts during ClO_2 Fumigation. A 2 × 2 × 2 factorial experiment was designed to investigate the major variables that might impact chlorate and perchlorate formation from chlorine dioxide gas in glass reaction chambers. Main factors were reagent matrix (liquid or dry), chlorine dioxide gas concentration (1.6 or 7.8 mg per 0.95 L), and the presence or absence of light. The complete experiment was replicated on each of four consecutive days for a total "*n*" of four observations for each treatment combination. Reactions were conducted in clear glass quart jars (0.95 L) in the absence of vegetable matter. Jars containing treatments protected from light were entirely covered with aluminum foil, whereas jars containing treatments exposed to light were left uncovered. The levels of chlorine dioxide gas (1.6 and 7.8 mg; nominal concentrations of 600 and 3000 ppmv) were selected to bracket the mass of chlorine dioxide used in radiolabeled experiments (5.5 mg) with tomatoes.

Experiments were conducted in a laboratory illuminated by indirect sunlight, electronically ballasted F28T8 fluorescent laboratory lights (28 W), and F40T12 magnetically ballasted fluorescent laboratory hood lights. With the laboratory and hood lights illuminated, the light intensity was 900 ± 17 lx (mean ± std dev; 54 observations on 6 separate days). With laboratory and hood lights on, overcast or sunny days did not significantly influence in-hood light intensity (P = 0.10).

Dry media chlorine dioxide generation employed FruitGard granules (ICA TriNova; Atlanta, GA). In the dry media experiments equal amounts of FruitGard chlorite impregnate and ICA dry acid activator impregnate were combined in Tyvek sachets or in glass beakers. The media were mixed by hand agitation to commence chlorine dioxide generation. Dry matrix reagents were sequentially and separately weighed (0.21 and 1.05 g of impregnate and activator, respectively, for the 1.6 and 7.8 mg ClO_2 treatments). Upon the addition of matrix part B, the contents of each sachet were mixed by hand agitation. Sachets were immediately placed in labeled, transparent 0.95 L canning jars and sealed with a canning jar lid and ring. The time at which each reaction was started was recorded.

Liquid reagents were added to Tyvek sachets in the sequence for 1.6 and 7.8 mg treatments respectively: nanopure water (96 or 529 μL) and technical grade sodium chlorite (10 or 52 μL; 318 mg/mL; ICA-TriNova; Atlanta, GA) followed by 1.8 M HCl (66 or 363 μL). After the addition of HCl, sachets were mixed by hand agitation and immediately placed into labeled, transparent 0.95 L canning jars and jars sealed. The start time was then recorded.

Reactions were allowed to proceed for 2 h, after which each container was unsealed and vented into the fume hood. Sachets were removed and each jar was rinsed sequentially by vigorously shaking four 50 mL aliquots of nanopure water within the sealed jars. Water rinses were transferred to labeled 250 mL volumetric flasks, the flasks were diluted to volume, and each flask was mixed by inverting a

minimum of 10 times. Aliquots of each sample were placed in labeled containers and were frozen until analysis by ion chromatography.

Perchlorate analyses were conducted using a Thermo-Fisher ICS-2100 ion chromatograph using the framework outlined in EPA method 314.0.[15] Sample aliquots (1 mL) were injected onto a Dionex AS16-HC column (4 × 250 mm) protected by an AG16-HC guard column (4 × 50 mm). An isocratic mobile phase of 50 mM KOH, produced using a Thermo-Fisher eluent generator, with a flow rate of 1.5 mL/min was used to elute perchlorate from the column. Perchlorate was measured using suppressed conductivity detection in-line with external water (ASRS 300) suppression (186 mA). Sodium perchlorate standards (4, 10, 25, 100, 200, and 400 μg/L), prepared in nanopure water, were injected in replicate 1 mL aliquots onto the ion chromatograph. Peak areas were regressed against perchlorate concentration using Chromeleon CHM-2 software (Thermo-Fisher). The LOQ was equivalent to the lowest standard (4 μg/L). The LOD of 0.001 μg/L was determined empirically by injecting replicate 1 mL aliquots of 0.5, 1, and 4 μg/L sodium perchlorate standards.

Chlorate analyses were also conducted using a Thermo-Fisher ICS-2100 ion chromatograph. Standards consisting of 5, 25, 100, 500, 1000, and 5000 μg/L of sodium chlorate were prepared in nanopure water. Chlorate was separated from interferences on a 4 × 250 mm Dionex AC19HC column protected by a 4 × 50 mm AS19HC guard column with an isocratic mobile phase of 20 mM KOH. Mobile phase was prepared using a Thermo Fisher eluent generator. Ions were detected using a DS6 conductivity detector with external water suppression (ASRS 300; 50 mA). Sodium chlorate standards were run at the beginning and end of each sample set. Blank samples were also concurrently run with each analysis. For the chlorate analysis, the LOQ was 5 μg/L; the LOD of 1 μg/L was determined empirically.

Statistics. SigmaPlot 12.0 (Systat Software, Inc.; San Jose, CA) was used to determine differences in treatment (dry or liquid reagent; chlorine dioxide concentration; or illuminated or dark fumigation) means for perchlorate and chlorate. Main effects of media and chlorine dioxide concentration on chlorate and perchlorate formation were determined using two-way analysis of variance (ANOVA) after passing tests of normality and equal variance. The effect of light was not included in the analyses because of the low to nondetectable levels of chlorate and perchlorate in samples protected from light. Chlorate residues were log-transformed prior to statistical analyses in order to meet the equal variance assumption. The Holm−Sidak method was used to determine differences in treatment means after the 2-way ANOVA F statistic indicated significant main effects. F statistics of less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Fate of ^{36}Cl-Chlorine Dioxide in a Tomato Fumigation System. Table 2 shows the distribution of radioactivity in tomato fumigation systems after 2 h fumigations with $^{36}ClO_2$. Across trials approximately 12% of the system radioactivity was present on the tomato itself; 2 to 13% was rinsed from the fumigation tank and its associated parts (stir bar, glass pedestal); 15 to 19% was purged from the tank and trapped into sodium thiosulfate; and 44 to 49% was associated with the reaction sachet itself. Total recovery of radioactivity was 80 to 84%. Potential losses of radiochlorine include losses realized immediately after the acid activation of the reaction while the sachet was positioned in the reaction chamber and while sealing the chamber. Additional losses could have occurred during chamber evacuation by chlorine dioxide absorption or condensation on the Tygon tubing that transported gases from the reaction chamber to the thiosulfate trap or during the fumigation period by leakage from the tank. Of the total system radioactivity, approximately 55.9% was converted to ^{36}Cl-ClO_2 (total starting radioactivity less activity remaining in the sachet); thus, of the total chlorine dioxide formed, approximately 22% was associated with the tomato itself.

Of note with regard to the gross disposition of the radioactivity was the apparently smaller amount of radioactivity that was rinsed from the tank wall, lid, pedestal, and stir rod in trial C (1.6%) compared to tank rinses of trials A and B (11 and 13%, respectively). Gases in trial C were protected from light, compared to trials A and B. Because the dark-phase radioactive experiment was not replicated, it would be unwise to make too many inferences from a single observation.

Tomato radioactive residues were the collective residues present in the tomato rinsewater, in the tomato puree, and in the stem scar region (for trials B and C). For trial A, 11.9% of the total starting activity was associated with the tomato after a 2 h exposure period, with 13.7 and 11.0% of the starting radioactivity associated with the tomatoes of trials B and C, respectively. While the surface area of the tomato was small relative to the surface area of the tank and its components, $^{36}ClO_2$ deposition was disproportionately associated with tomatoes. That is, approximately 56% of the total starting radioactivity was converted to $^{36}ClO_2$, with 20−25% of the $^{36}ClO_2$ radioactivity associated with the tomato. The disproportional deposition of radioactivity onto the tomato is not surprising given the water solubility of chlorine dioxide and the expected availability of reducing agents in a tomato matrix. The attraction of chlorine dioxide to the tomato itself also provides context for its efficacy at killing pathogens[16−18] and rot organisms[9,19] on vegetable surfaces.

Between 1.1 and 2.2% of the total system radioactivity was rinsed from the surface of tomatoes after the 2 h exposure. When expressed as the total activity present on the tomato, the rinse contained 10 (trial C) to 16% (trials A and B) of the tomato TRR. Data from trials B and C clearly show that the stem scar region of the tomato preferentially accumulated radioactive residues. For example, the stem scar from trial B contained 28.5% of the total activity associated with the tomato even though the scar region was only 0.64 g; for trial C, the scar area contained 20% of the total tomato activity, while comprising only 0.24 g of the total tomato mass. Given the low mass and the high concentration of TRR, one would expect that chlorine dioxide would have high efficacy at the moist, porous area of the fruit, those regions in which pathogens and rot organisms might have the highest probability of colonizing.

Figure 2 shows example chromatograms of sodium chlorite, sodium chloride, and sodium chlorate standards in water and a representative chromatogram of tomato rinsewater assayed for radiochlorine content by ion chromatography with subsequent trapping of radioactive fractions. Using the AS11-HC column chlorite, chloride, and chlorate were well resolved. The large peaks shown in Figure 2 are a reflection of the fact that each aliquot of tomato rinse chromatographed was fortified with unlabeled standards to assist in accurate trapping. Across trials, radioactive sodium chlorite was typically not present in rinse fractions; in contrast, the sodium chloride and chlorate fractions contained detectable radioactivity across trials. Total recovery of radioactivity was low (generally less than 70%) when sample aliquots were analyzed on the AS11-HC column so sample aliquots were also analyzed using an AS16-HC column which allowed perchlorate elution. Using the AS16-HC column, perchlorate was well resolved from chlorite, chloride, and chlorate, which coeluted (Figure 3).

Table 3 shows the composition of radioactive residues in tomato fractions of trials A, B, and C. Tomato rinse fractions did not contain detectable sodium chlorite in trials A and B, but did contain detectable (0.01 μg/g of tomato) ^{36}Cl-chlorite in

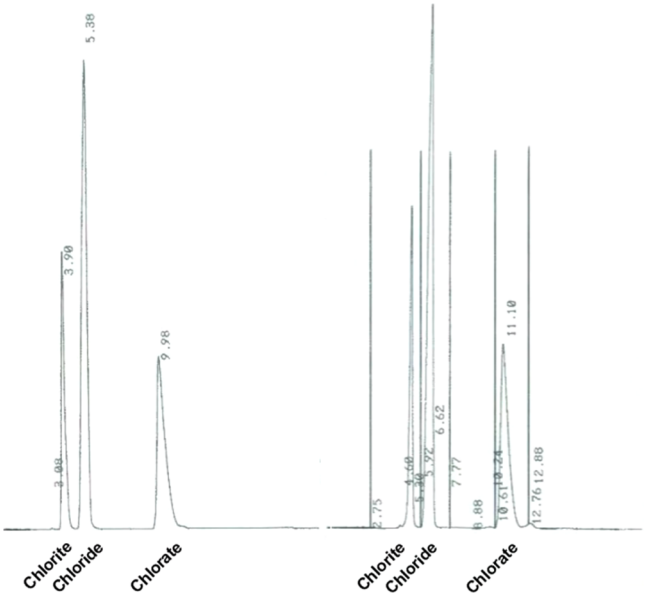

Figure 2. Ion chromatographic separation of unlabeled chlorite, chloride, and chlorate standards in water (10 μL; left panel), and an aliquot (1000 μL) of tomato rinse fortified with the standard mix (right panel). Vertical lines in the chromatogram of the tomato rinse fraction represent the regions collected directly into LSC vials for determining radioactive residues. Radioactivity was never present in the chlorite fraction of the tomato rinse, but it was significantly above background for the chloride and chlorate fractions in the tomato rinse aliquot. Differences in retention times of standards in the two injections are a function of the differing injection volumes at a constant flow rate (1 mL/min).

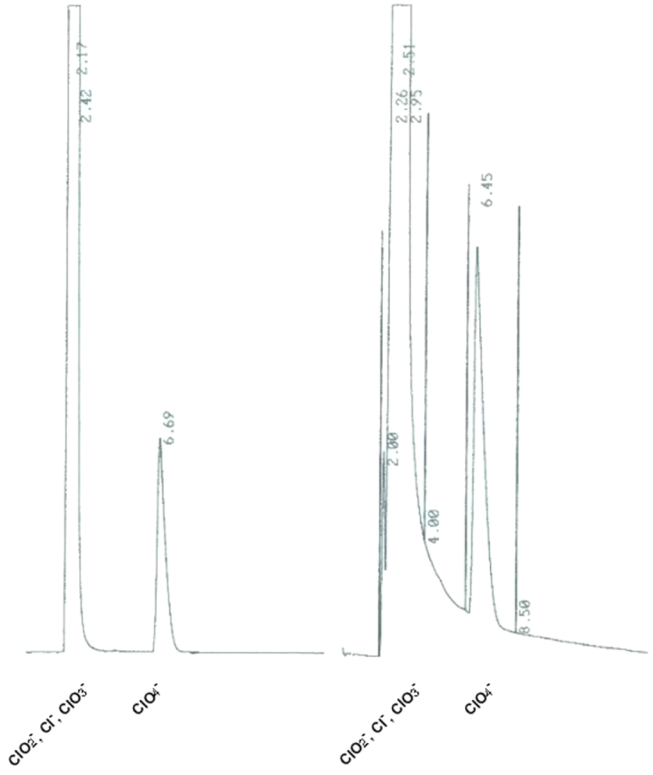

Figure 3. Ion chromatographic separation of unlabeled chlorite, chloride, chlorate, and perchlorate standards (left panel) and an aliquot of tomato serum containing incurred residue (right panel) and fortified with the standard mix. Radioactivity present as chlorite, chloride, and chlorate was not resolved on the AS16-HC column and was trapped together; perchlorate was trapped as a single peak, well resolved from chlorite, chloride, and chlorate. Vertical lines in the chromatogram of the tomato rinse fraction represent the regions collected directly into LSC vials for determining radioactive residues.

one of three sample aliquots measured from trial C. Sodium chlorate was the major residue rinsed from tomatoes of trials A and B (56.5 to 67.5% of total residue) but represented 10% of the total residue in trial C, which was run under dark conditions. Similarly, when reactions were run under laboratory illumination (trials A and B), ^{36}Cl-perchlorate represented significant quantities of radioactivity in the tomato rinse (8.2 to 17.7% of rinse activity), but under conditions protected from light, no detectable ^{36}Cl-perchlorate was present (trial C; LOQ of 4 ng/mL using ion chromatography). Radioactive chloride ion present on tomato surfaces was greatly influenced by illumination during the experiment, with trial A and B tomato rinses containing 14.8 to 35.2% chloride and trial C (dark) TRR being composed of 89.1% ^{36}Cl-chloride. Thus, the major factor affecting the composition of residues rinsed from the surface of tomatoes was whether fumigations were exposed to laboratory light.

Of the total radioactive residues present in tomato puree, 53.9 ± 1.6 of the activity partitioned into the serum, while 46.1% partitioned into the pulp. Tomato serum, prepared from tomato puree, contained no detectable sodium chlorite (Table 3). Radioactive chloride ion represented 80 to 87% of the serum radioactivity in illuminated fumigations (trials A and B) and 93% of the serum radioactivity in the darkened fumigation (trial C). In illuminated fumigations, chlorate represented 13 to 19% of the serum activity, with the proportion dropping to 5% of the total activity for trial C serum (dark). Perchlorate was not consistently detected in sera of tomatoes, regardless of trial (LOQ 0.07 to 0.17 μg/g).

Stem scar radioactivity, measured in trials B and C, was composed primarily of sodium chloride (86 to 90% of total) and chlorate (9 to 13% of scar radiochlorine); perchlorate residues comprised about 1% of the total stem scar radiochlorine. It should be recalled, however, that the stem area of the tomato, while representing less than 1% of the tomato weight, contained 20 to 25% of the total radioactivity rinsed from, or deposited onto, the tomatoes.

Pellet radioactivity could not be measured directly via ion chromatography, so pellet aliquots were digested in NaOH and then reacted with an excess of silver nitrate to precipitate the ^{36}Cl-chloride ion. It was assumed that radioactivity not precipitated by silver nitrate had the same chemical composition as stem scar. For trials A and B, 91 to 100% of the radioactivity released by NaOH digestion was precipitated by silver nitrate, indicating that the released radiochlorine was ^{36}Cl-chloride ion. For trial C, 100% of the radioactivity was released by NaOH digestion, but only 97% of this radiochlorine was precipitated as ^{36}Cl-chloride ion.

From a qualitative perspective, residues rinsed from the surface of the glass reaction chamber were similar to residues rinsed from tomato surfaces, with laboratory illumination being the major influence on the composition of residues. Oxidized products of chlorine dioxide present in tank rinsewater from trial A represented a major amount of residue (95.3% of tank rinse residue), but for trial C (dark) the major residue was chloride (78.1% of tank rinse activity) with only 13.9% of the radioactivity being present as chlorate or perchlorate. The presence of light greatly influences the propensity of radioactive

Table 3. Composition of Radioactive Residues in Tomato Fractions and Tank Rinses of Tomato Trials A, B, and C

		residue composition and concn[a]						LOQ[b]
		trial A		trial B		trial C		trial A, B, C
fraction	residue[c]							
		%	μg/g	%	μg/g	%	μg/g	μg/g
tomato rinse	$Na^{36}ClO_2$	0	<0.01	0	<0.01	0.8[d]	<0.01[d]	0.01, 0.01, 0.01
	$Na^{36}Cl$[e]	35.2	0.22	14.8	0.11	89.1	0.32	0.01, 0.003, 0.01
	$Na^{36}ClO_3$	56.5	0.65	67.5	0.92	10.1	0.7	0.01, 0.01, 0.005
	$Na^{36}ClO_4$	8.2	0.11	17.7	0.28	0	NDR[f]	0.02, 0.02, 0.004[f]
serum	$Na^{36}ClO_2$	0	<0.080	0	<0.05	0	<0.08	0.08, 0.05, 0.08
	$Na^{36}Cl$[e]	86.7	6.5	79.6	3.1	93.2	3.6	0.05, 0.03, 0.05
	$Na^{36}ClO_3$	12.8	1.8	19.1	1.3	5.4	0.4	0.09, 0.06, 0.09
	$Na^{36}ClO_4$	0.5[g]	<0.17[g]	1.3[g]	<0.17[g]	1.4[h]	0.1[h]	0.17, 0.17, 0.07
stem scar	$Na^{36}ClO_2$			0	<2.2	0.7	8.0	[ ], 2.2, 6.1
	$Na^{36}Cl$[e]			86.2	444.1	89.5	685	[ ], 1.4, 6.7
	$Na^{36}ClO_3$			12.5	116.9	9.0	125	[ ], 2.6, 4.3
	$Na^{36}ClO_4$			1.1	11.9	0.8	12	[ ], 5.4, 7.9
pellet	$Na^{36}ClO_2$	0	<0.43	0	<0.65	0.2	<0.86	0.43, 0.65, 0.86
	$Na^{36}Cl$[e]	100[i]	6.0	91.0[j]	12.8	97.3[k]	25.9	0.28, 0.42, 0.56
	$Na^{36}ClO_3$	0	<0.51	8.3	2.1	2.3	1.13	0.51, 0.76, 1.02
	$Na^{36}ClO_4$	0	<0.58	0.7	<0.88	0.2	<1.17	0.58, 0.88, 1.17
		%	μg/mL	%	μg/mL	%	μg/mL	μg/g
tank rinse	$Na^{36}ClO_2$	0.4[h]	0.01			8.0	0.01	0.01, [ ], 0.008
	$Na^{36}Cl$[e]	3.7	0.08			78.1	0.08	0.007, [ ], 0.005
	$Na^{36}ClO_3$	82.5	3.14			7.2	0.01	0.012, [ ], 0.009
	$Na^{36}ClO_4$	13.4	0.59	31.0	1.10	6.7	0.01	0.017, 0.031, 0.007

[a]Concentration in fraction; calculated by dividing the mass of residue by the fraction wt. (μg ÷ g = μg/g). [b]Limit of quantitation; based on the background radioactivity that was determined with each sample set and also based on the sample aliquot size used. [c]Based on the ion chromatographic separation of ^{36}Cl-chlorite, ^{36}Cl-chloride, ^{36}Cl-chlorate, and ^{36}Cl-perchlorate with determination of radioactivity in trapped fractions by liquid scintillation counting. [d]Radioactivity was detected and quantified in only 1 of 3 replicates. Thus, the calculated concentration is less than the limit of quantification. [e]Sodium chloride derived from $^{36}ClO_2$ gas; residues do not account for endogenous sodium chloride. [f]NDR, no detectable residue; perchlorate concentration in trial C rinsewater was also determined using ion chromatography having an LOQ of 4 ng/L. [g]Analyte was detected in one of three replicates, thus the calculated concentration is less than the LOQ. [h]Analyte was detected in two of three replicates. [i]98.6% of the pellet radioactivity was released by digestion in NaOH; of the released radioactivity, 100% was precipitated with silver nitrate. [j]97.3% of the pellet radioactivity was released by digestion in NaOH; of this, 91% was precipitated with silver nitrate (indicating chloride ions); it was assumed that the remaining 9% of the radioactivity was chlorate and perchlorate in the same proportion as in the stem scar. [k]100% of the pellet radioactivity was released by digestion in NaOH; of this, 97.3% was precipitated with silver nitrate (indicating chloride ions); it was assumed that the remaining 2.7% of the radioactivity was chlorate and perchlorate in the same proportion as in the stem scar.

residue to be deposited on the tank surface (Table 1), with light exposed chamber rinses containing 10.1 to 12.5% of the total starting activity; only 0.6% of the starting radioactivity in the chamber protected from light was deposited on the tank surface.

Fate of Sodium ^{36}Cl-Chlorite Injected into Tomatoes. We hypothesized that instability of sodium chlorite in weak acids[20] would make it virtually impossible for chlorite residues to survive the acidity of tomatoes during processing. This hypothesis was tested by directly injecting sodium ^{36}Cl-chlorite into tomatoes and subsequently following the chemical fate of the radiolabel. The exposure level selected (131 μg/g of tomato) was chosen to greatly exaggerate concentrations of chlorite in an anticipated commercial fumigation, even if 100% of a chlorine dioxide fumigation were to be converted to chlorite residue.

Radioactive residues injected into tomatoes distributed primarily to the serum fraction (87.3%) after centrifugation with only 13.7% of the activity distributing to the tomato solids. Radioactivity associated with chloride ion represented 98.3% the serum activity, with chlorate ion composing the complement. No chlorite ion was detected, indicating that 100% of the starting chlorite had been consumed. The qualitative (formation of chloride and chlorate) results are consistent with the aqueous degradation of sodium chlorite in the presence of organic acids.[21]

Fate of ^{36}Cl-Chlorine Dioxide in a Cantaloupe Fumigation System. *Disposition of Radioactive Residues.* Table 4 shows the distribution of radioactivity expressed as total dpm in each fraction and as the percentages of the total starting radioactivity for cantaloupe trials A and B. Table 4 also shows the initial cantaloupe weights, the weights of the edible flesh, seed bed, and inedible rind fractions, and the total recovery of weight for each melon. The total recovery of radioactivity was comparable between cantaloupe trials A (89.4%) and B (88.3%). Unrecovered radioactivity (10.6 and 11.7% of the total for trials A and B, respectively) likely resulted from two factors. First, some $^{36}ClO_2$ was almost certainly lost to the atmosphere between sachet activation, positioning in the exposure tank, and sealing the tank. Additionally, $^{36}ClO_2$ may have been lost because of incomplete gas purging at the end of the treatment period or leakage during fumigation.

The total amount of released $^{36}ClO_2$ was calculated as the difference between the total starting activity and the radioactivity recovered in, and on, the reaction sachet. Therefore, about 63% of the starting activity was associated with the

Table 4. Distribution of Radioactivity after the Fumigation of Cantaloupe with $^{36}ClO_2$ Gas

	trial A				trial B			
	wt[a]		act.[b]		wt[a]		act.[b]	
item	g	%	dpm	%	g	%	dpm	%
starting amt								
melon	851.6	100.0			850.0	100.0		
total act.[c]			22,270,000	100.0			22,270,000	100.0
$Na^{36}ClO_2$			20,150,000	90.5			20,150,000	90.5
$Na^{36}Cl$			2,115,000	9.5			2,115,000	9.5
residues from $^{36}ClO_2$ exposure[d]								
edible flesh puree	325.8	38.3	<LOD	0	294.9	34.7	<LOD	0.0
seed bed puree	62.4	7.3	3,532	<0.1	49.3	5.8	4,669	<.1
rind puree	458.5	53.8	7,225,000	32.5	500.9	58.9	8,331,000	37.4
recovery, melon	846.7	99.4	7,229,000	32.5	845.1	99.4	8,336,000	37.4
chamber gas purge			4,059,000	18.2			2,328,000	10.5
chamber rinse			382,000	1.7			636,900	2.9
recovery, chamber			4,441,000	19.9			2,965,000	13.3
sachet activity (non $^{36}ClO_2$)[e]								
rinse			8,111,000	36.4			8,235,000	37.0
bound			124,000	0.6			132,000	0.6
recovery, sachet			8,235,000	37.0			8,367,000	37.6
total recovery	846.7	99.4	19,900,000	89.4	845.1	99.4	19,670,000	88.3
unrecovered (gas phase)[f]			2,361,000	10.6			2,598,000	11.7
total gas phase[g]			14,030,000	63.0			13,900,000	62.4

[a]Weight of melon and melon fractions. [b]Radioactive residues in indicated fraction, percentage of starting radioactivity. [c]Total amount of radiochlorine added to the Tyvek sachet; radiochemical purity of the sodium ^{36}Cl-chlorite was 90.5% [d]Radioactive residues present on the melon fractions, in the chamber gas purge, and on the chamber walls, glass stand, and stir bar could only occur through the production of $^{36}ClO_2$ gas. [e]Sachet activity; residual radioactivity that did not exit the Tyvek sachet as chlorine dioxide gas. [f]Radioactivity not present in the melon residues, chamber gas purge or rinse, and sachet. [g]Sum of "recovery, melon", "recovery, chamber"; and "unrecovered (gas phase)" items.

Table 5. Speciation of Radioactivity Present in Cantaloupe Edible Flesh, Inedible Rind Serum, and Tank Rinse Fractions of Cantaloupe Trials A and B

		residue composition and concn[a]				
		trial A		trial B		LOQ[b] A, B
fraction	residue[c]					
		%	μg/g	%	μg/g	μg/g
edible flesh	TRR[d]	0.0	NDR[e]	0.0	NDR	0.4, 0.3[f]
rind serum	$Na^{36}ClO_2$	0.0	<0.8	0.0	<1.0	0.8, 1.0
	$Na^{36}Cl$[g]	86.3	74.4	87.0	69.6	0.5, 0.6
	$Na^{36}ClO_3$	13.7	21.6	12.3	18.0	0.9, 1.2
	$Na^{36}ClO_4$	0.0	<0.3	0.7	1.2	0.3, 0.7
		%	μg/mL	%	μg/mL	μg/mL
tank rinse	$Na^{36}ClO_2$	0.0	<0.1	0.0	<0.1	0.11
	$Na^{36}Cl$[g]	11.3	0.3	6.2	0.3	0.07
	$Na^{36}ClO_3$	55.5	2.5	79.7	6.0	0.13
	$Na^{36}ClO_4$	33.3	1.7	14.1	1.2	0.03, 0.07

[a]Concentration in fraction; calculated by dividing the mass of residue by the fraction wt. (μg ÷ g = μg/g). [b]Limit of quantitation; based on the background radioactivity that was determined with each sample set and also based on the sample aliquot size used. [c]Based on the ion chromatographic separation of ^{36}Cl-chlorite, ^{36}Cl-chloride, ^{36}Cl-chlorate, and ^{36}Cl-perchlorate with determination of radioactivity in trapped fractions by liquid scintillation counting. [d]TRR, total radioactive residue. [e]NDR, no detectable residue. [f]LOQ for total radioactive residues assumes all residue is present as sodium chlorate equivalents. [g]Sodium chloride derived from $^{36}ClO_2$ gas; residues do not account for endogenous sodium chloride.

formation of $^{36}ClO_2$ gas in trials A and B. The specific activity of $^{36}ClO_2$ for each trial was 135 dpm/μg; therefore 104 and 103 mg of $^{36}ClO_2$ were produced in trials A and B, respectively. The cantaloupe weights used for trials A and B were 851.6 and 850.0 g, respectively, corresponding to ClO_2 exposures of 123 and 122 mg/kg of cantaloupe. Because *maximal* commercial exposures to chlorine dioxide are expected to be 100 mg of chlorine dioxide per kg of cantaloupe, data presented in this study represent residue levels commensurate with 120% over exposure relative to expected.

Cantaloupes retained 32.5 and 37.4% of the total radioactive charge in trials A and B (Table 4), respectively, representing 51.5 and 60.0% of the total $^{36}ClO_2$ produced from each reaction. Of the TRR present on the cantaloupe, greater than 99.97% of the cantaloupe radiochlorine was associated with the rind, regardless of trial (Table 4). Radioactive residues present

Table 6. Production of Chlorine Dioxide Degradation Products in Rinses of Jars Treated with 1.6 or 7.8 mg of Chlorine Dioxide Using Dry or Liquid Reagent Matrices and in the Presence or Absence of Light[a]

	light			dark	
	μg/L			μg/L	
level of chlorine dioxide[b]	dry matrix	liquid matrix	*P*	dry matrix	liquid matrix
		Perchlorate[c]			
low: 1.6 mg	456 ± 233	438 ± 98	0.96	NDR[d]	NDR
high: 7.8 mg	4334 ± 838	880 ± 211	<0.01	NDR	NDR
P	<0.01	0.19			
		Chlorate[e]			
low: 1.6 mg	998 ± 58	2,734 ± 289	<0.01	11[f]	<LOQ[g]
high: 7.8 mg	4,858 ± 693	17,497 ± 837	<0.01	NDR	11 ± 2
P	<0.01	<0.01			

[a]Data are means ± standard deviations of four observations unless indicated by a footnote. NDR signifies no detectable residue. Statistical inferences of chlorate residues generated under lighted conditions were generated using log-transformed data (to meet the equal variance assumption). [b]Nominal concentrations of 600 and 3000 ppmv for the 1.6 and 7.8 mg reactions, respectively. [c]LOQ, 4 μg/L; LOD, 1 μg/L. Data are expressed on a sodium perchlorate equivalent basis. [d]Three of four replicates had no detectable residue; a single replicate had residues >LOD but less than the LOQ. [e]LOQ, 5 μg/L; LOD, 1 μg/L. Data are expressed on a sodium chlorate equivalent basis. [f]Single observation; remaining replicates had NDR. [g]All replicates had chlorate residues less than the LOQ, but greater than the LOD.

in the edible flesh portion of the cantaloupe were below the LOQ of the radiochemical assay (0.240 to 0.360 μg/g of sodium chlorite equivalents). Total radioactive residues present in seed beds represented 0.03% of the total radioactivity produced as $^{36}ClO_2$. Activity removed from the surfaces of the exposure tank, glass pedestal, and stir bar accounted for 1.7% of the total radioactivity in trial A and 2.9% in trial B. Gas purged from the exposure tank represented 18.2% of the initial activity for trial A and 10.5% for trial B. The sachet and its contents contained 37.0 and 37.6% of initial activity respectively for A and B.

Radioactive residues were not detected in edible cantaloupe flesh, indicating that chlorine dioxide gas does not penetrate the rind and acts at the cantaloupe surface. In the inedible rind fraction, radioactivity was nearly equally distributed between the rind serum (57.4 and 45.2% of rind radioactivity in trials A and B, respectively) and pellet fractions (42.6 and 54.8% of rind radioactivity in trials A and B, respectively) even though the serum fraction was the greatest by weight. Because the serum fractions represented 64 to 80% of the total rind fraction, relative concentrations of radioactivity were greater in the rind pellet fractions than in the rind serum fractions.

Speciation of Radioactive Residues. Table 5 summarizes residues of ^{36}Cl-chlorite, ^{36}Cl-chloride, ^{36}Cl-chlorate, and ^{36}Cl-perchlorate measured in fractions containing sufficient radioactive residues for speciation: rind serum and tank rinse fractions for both trials A and B. No sodium ^{36}Cl-chlorite was detected in inedible rind serum or tank rinse, whereas sodium ^{36}Cl-chloride was the predominant $^{36}ClO_2$-derived residue on cantaloupe rind comprising 86 to 87% of the rind radioactivity. The only other non-chloride residue on cantaloupe rind of trial A was sodium ^{36}Cl-chlorate representing about 13% of the rind TRR with sodium ^{36}Cl-perchlorate being nondetectable in rind serum of trial A, but representing 0.7% of the rind radioactivity in trial B (1.2 μg/g). In the tank rinse fraction, sodium ^{36}Cl-chlorate and sodium ^{36}Cl-perchlorate were the predominant radioactive residues, with sodium chlorate representing 56 to 80% of the total residue, and sodium perchlorate representing 14 to 33% of the total residue with sodium chloride a minor radioactive residue, representing 6 to 11% of the TRR. No effort to protect the cantaloupe exposures from light was made. Subsequent experiments with nonlabeled chlorine dioxide have demonstrated that chlorate and perchlorate formation during fumigation of cantaloupe can be essentially eliminated by protecting the reaction from light.

It is notable that the percentage compositions of radioactive residues on the cantaloupe rind and tank rinse fractions were not very similar. On the rind, sodium chloride was, by far, the major chlorine dioxide degradation product, representing between 86 and 87% of the radioactivity, with chlorate representing essentially the balance of activity. Such results are consistent with chlorine dioxide reductive processes and aqueous disproportionation reactions.[21,22] In contrast, glass surfaces contained mainly chlorate (55 to 80% of total glass rinse residue) and perchlorate (14 to 33% of total glass rinse residue), with lesser quantities of sodium chloride (6 to 11% of rinse radioactivity). The formation of mainly perchlorate and chlorate on glass surfaces is consistent with light catalyzed gas-phase reactions.[23,24] The formation of perchlorate from chlorine dioxide gas generated under a number of conditions is light dependent.[25,26]

As stated earlier, puree of edible flesh had no detectable residues in either trial A or B with LOD/Qs below 0.5 μg/g for sodium chlorite, sodium chlorate, and sodium perchlorate. Because it was reasoned that radioactive residues might concentrate in either the solid or liquid portions of the edible flesh, the puree was centrifuged to form liquid (serum) and pellet fractions. When serum was assayed, radioactive residues were detected in trial B cantaloupe edible flesh serum, but not in serum from trial A. Because a 1 g sample size was used to assay serum from trial B, a lower detection limit (<0.12 μg/g for each of sodium chlorite, sodium chlorate, and sodium perchlorate) was obtained. Precipitation of radioactive residues present in trial B serum with silver nitrate caused a 90% loss of activity from the serum, indicating that at least 90% of the edible flesh serum was sodium ^{36}Cl-chloride. Assuming that the remaining 10% of the radioactive residue in edible flesh serum of trial B was either sodium ^{36}Cl-chlorate or sodium ^{36}Cl-perchlorate, then the concentration of radioactivity, expressed as sodium chlorate or sodium perchlorate equivalents, in serum would be 0.017 or 0.019 ng/g, respectively.

Factors Impacting the Formation of Chlorate and Perchlorate Byproducts from Chlorine Dioxide Fumigation. This experiment was conducted with a balanced factorial design

with level of chlorine dioxide, laboratory illumination, and reagent matrix (dry vs liquid) as main factors. Table 6 summarizes results of the experiment in which chlorate and perchlorate recovered in reaction jar rinsewater are expressed as μg/L of the sodium salt equivalents.

Data presented in Table 6 clearly demonstrate that light had a major impact on the formation of both perchlorate and chlorate from chlorine dioxide. Under dark conditions, insufficient perchlorate was formed to exceed the assay LOQ of 4 μg/L. Of the 16 samples excluded from light, perchlorate was formed in only two samples at levels that surpassed the assay LOD of 1 μg/L. In contrast, the reaction chambers exposed to light had mean perchlorate concentrations ranging from 438 to 4,334 μg/L, depending upon the amount of chlorine dioxide produced. In a similar manner, light catalyzed the formation of chlorate (means of 998 to 17,947 μg/L) on vessel walls; but under dark conditions, chlorate residues were either not detectable (LOD of 1 μg/L) or low, with a maximum concentration of 11 μg/L (Table 6). Statistical comparisons between means from dark and light exposed vessels were not possible because the darkened vessels contained insufficient chlorate and perchlorate for the calculation of treatment means (Table 6).

For the treatments exposed to light, however, significant ($P < 0.001$) main effects for both chlorine dioxide concentration and the reaction matrix were noted (Table 3). For perchlorate, a highly significant ($P < 0.001$) interaction between the target chlorine dioxide concentration and reaction matrix was observed, so no simple relationship existed. For chlorate, main effects of chlorine dioxide concentration ($P < 0.01$) and reaction matrix ($P = 0.02$) were significant, with the high chlorine dioxide concentration and liquid reaction matrix consistently producing greater quantities of chlorate than the low chlorine dioxide target concentration and the dry matrix.

The literature suggests that both light[23,24] and gas concentration[27] affect chlorine dioxide stability. Our data are also consistent with the notion that chlorine dioxide decomposition is catalyzed by light and (or) high gas concentrations. Spinks and Porter[23] reported the formation of perchlorate by gaseous chlorine dioxide decomposition, and the formation of perchlorate was dependent upon the presence of water vapor. Crawford and Dewitt[24] suggested that water vapor reacts with unstable chloroxy intermediates to form acid gases of chlorate and perchlorate. They also reported a wall to vessel volume relationship in the rate of reaction intermediate termination; presumably vessel walls act as terminal points for unstable radicals created during gas decomposition. Such data might explain the relatively high degree of deposition of ^{36}Cl-chorate and ^{36}Cl-perchlorate on vessel walls in which light exposure was not controlled, even with the presence of chlorine dioxide sinks (tomatoes or cantaloupe).

In the absence of light, but in the presence of water vapor, the terminal (i.e., stable) decomposition products of chlorine dioxide gas would be chlorate and chloride, consistent with the aqueous decomposition of chlorine dioxide.[22,28] Our data (Table 6), however, strongly suggest that, even in the presence of water vapor (i.e., liquid matrix), light must be present to catalyze the formation of chlorate (and also perchlorate). Thus, the mechanism for the formation of both perchlorate and chlorate is through the light catalyzed formation of unstable[25] intermediates such as chlorine perchlorate (Cl_2O_4).[26]

Collectively, the experiments reported herein clearly indicate the potential for chlorine dioxide fumigation of vegetables and melons from a residue chemistry perspective. Studies using radiolabel, for example, indicate that edible flesh of cantaloupe contains no chlorine dioxide related residue whatsoever. Additionally, for either tomato or cantaloupe, the major residue associated with vegetable matter is the chloride ion. Nevertheless, radiolabeled studies also show the potential for both chlorate and perchlorate formation during ClO_2 fumigation, especially if the fumigation is not protected from light. Further work is being conducted to determine fumigation conditions under which the formation of chlorate and perchlorate on vegetable matter may be minimized or prevented entirely. These studies will provide data supporting or refuting the concept that chlorine dioxide fumigation of produce can be accomplished without the formation of undesirable residual chloroxyanions.

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Chloroxyanion Residues in Cantaloupe and Tomatoes after Chlorine Dioxide Gas Sanitation

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Supporting Information

ABSTRACT: Chlorine dioxide gas is effective at cleansing fruits and vegetables of bacterial pathogens and(or) rot organisms, but little data are available on chemical residues remaining subsequent to chlorine gas treatment. Therefore, studies were conducted to quantify chlorate and perchlorate residues after tomato and cantaloupe treatment with chlorine dioxide gas. Treatments delivered 50 mg of chlorine dioxide gas per kg of tomato (2-h treatment) and 100 mg of gas per kg of cantaloupe (6-h treatment) in sealed, darkened containers. Chlorate residues in tomato and cantaloupe edible flesh homogenates were less than the LC−MS/MS limit of quantitation (60 and 30 ng/g respectively), but were 1319 ± 247 ng/g in rind + edible flesh of cantaloupe. Perchlorate residues in all fractions of chlorine dioxide-treated tomatoes and cantaloupe were not different ($P > 0.05$) than perchlorate residues in similar fractions of untreated tomatoes and cantaloupe. Data from this study suggest that chlorine dioxide sanitation of edible vegetables and melons can be conducted without the formation of unwanted residues in edible fractions.

KEYWORDS: *chlorine dioxide, chlorate, perchlorate, chlorite, residue, food safety*

INTRODUCTION

Chlorine dioxide (ClO_2) gas is a strong oxidizer that is highly effective at inactivating bacterial pathogens[1] and spores,[1] amoeba,[2] fungi,[3,4] rot organisms,[1] viruses,[5,6] and even insects.[7] In the United States, aqueous-based chlorine dioxide disinfectants and sanitizers have been approved by the US EPA for a diverse number of farm, bottling plant, and food processing, handling, and storage applications[8] including fruit and vegetable washes, flume water disinfection, meat and poultry treatment, food processing plant disinfection, water sanitation, odor control, medical waste disinfection, and municipal water treatment. Gaseous chlorine dioxide is approved as a sterilant for a variety of manufacturing and laboratory applications including the treatment of environmental surfaces, tools, and clean rooms. The gas is also used for odor control in a variety of settings. Chlorine dioxide gas has advantages over aqueous formulations because of its rapid diffusion, ease of mixing with air, and especially its ability to penetrate porous surfaces.[5,9]

Although efficacy of the gas against specific zoonotic and plant pathogens, including *Listeria monocytogenes*,[10,11] *E. coli* O157:H7,[12] and *Salmonella enterica*[13] is generally well-known, the use of gaseous chlorine dioxide on vegetables is not authorized by regulatory agencies. The major obstacle precluding regulatory approval for vegetable applications has been the lack of data describing chlorine dioxide's fate and chemical disposition on sanitized crop groups. To this end, Trinetta et al.[14] studied the fate of ClO_2 gas after surface application to tomatoes, oranges, apples, strawberries, lettuce, alfalfa sprouts, and cantaloupe using a colorimetric assay for ClO_2 and an ion chromatographic method for ions including chlorite, chlorate, and chloride. Whereas they concluded that "chlorine dioxide technology leaves minimal to no detectable chemical residues. . .", they did find extremely high concentrations of some chloroxyanions (chlorate on alfalfa sprouts exceeding 18 000 ppm, and neary 800 ppm chlorite on lettuce, for example) in water rinses collected the day of fumigation. Trinetta et al.[15] also used sufficiently high concentrations of gas during 10 min exposures to cause "significant discoloration, browning, and bleaching, due to gas treatment" on produce containing high concentrations of residue. Others[11,16,17] have also reported chlorite residues of greater than 1 mg/kg on strawberries and lettuce rinses subsequent to treatment with excess chlorine dioxide gas. An alternative approach to chlorine dioxide sanitation of produce involves longer duration (hours) treatment with fairly low chlorine dioxide gas concentrations using technology that provides a defined release of chlorine dioxide over time. Residues remaining on sanitized produce under mild treatment conditions have not been previously investigated.

Our laboratory has investigated the fate and disposition of radiolabeled chlorine dioxide gas ($^{36}ClO_2$) on tomatoes (50 mg/kg) and cantaloupe (100 mg/kg) during 2-h treatment periods.[18] The studies clearly indicated that radioactivity from $^{36}ClO_2$ (g) treatment was deposited on the surfaces of vegetable matter, especially on moist surfaces such as stem scars. The data also indicated that radioactive residues were not present in edible flesh of cantaloupe after $^{36}ClO_2$ treatment, but that ample residue was present on cantaloupe rind. Thus,

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Table 1. Temperature and Relative Humidity Measurements during Chlorine Dioxide Sanitation of Tomatoes and Cantaloupe

	tomato status			cantaloupe		
	present		absent	present		absent
time	(−) ClO_2	(+) ClO_2	(+) ClO_2[a]	(−) ClO_2	(+) ClO_2	(+) ClO_2
min	temperature °C					
0	20.3 ± 1.9	20.8 ± 1.0	23.5 ± 1.4	19.7 ± 1.5	15.7 ± 2.1	19.7 ± 1.2
30	19.0 ± 1.4	20.3 ± 0.9	23.4 ± 1.3			
60	18.9 ± 1.3	20.3 ± 0.9	23.4 ± 1.2	18.3 ± 1.2	16.0 ± 1.7	19.3 ± 0.6
90	19.0 ± 1.3	20.4 ± 0.8	23.4 ± 1.2			
120	19.2 ± 1.2	20.5 ± 0.8	23.3 ± 1.1	19.7 ± 0.6	16.7 ± 1.2	19.0 ± 0.0
240				20.7 ± 1.5	18.3 ± 1.5	19.3 ± 1.2
360				21.7 ± 1.5	20.0 ± 1.7	19.3 ± 1.2
	relative humidity (%)					
0	28 ± 1.2	33 ± 2.0	24 ± 0.7	42 ± 7.4	55 ± 8.7	37 ± 5.1
30	42 ± 1.0	44 ± 2.1	25 ± 0.0			
60	45 ± 1.5	47 ± 1.7	26 ± 1.4	64 ± 1.5	69 ± 0.6	48 ± 2.3
90	48 ± 1.5	50 ± 1.7	29 ± 0.7			
120	51 ± 2.1	53 ± 2.1	30 ± 0.0	69 ± 1.5	72 ± 1.0	49 ± 2.6
240				77 ± 2.0	78 ± 1.7	49 ± 2.6
360				84 ± 1.5	82 ± 1.2	49 ± 2.3

[a]Means of two replicates for the tomato study; the temperature and humidity were not measured on a single replicate of tomatoes.

gaseous sanitation with $^{36}ClO_2$ was a surface phenomenon. Kaur et al.,[19] using a higher specific activity label than Smith et al.,[18] generated essentially identical results: $^{36}ClO_2$ treatment resulted in substantial total radioactive residue on cantaloupe surface, but nondetectable radioactive residues in the edible flesh portion of the melon.

Also clearly established by tracer studies using tomatoes[18] and cantaloupe[18,19] is that the most prevalent chemical residue related to $^{36}ClO_2$ (g) exposure was chloride ion (Cl^-), a ubiquitous nutrient which from a food-safety standpoint can be ignored. Chloride is the five electron reduction product of ClO_2 gas. Under certain conditions, however, two chloroxyanion byproducts, chlorate[18,19] (ClO_3^-) and perchlorate[18] (ClO_4^-) may also form during chlorine dioxide sanitation. The formation of chlorate and perchlorate can be minimized or essentially eliminated, however, if chlorine dioxide sanitation processes are protected from light.[18] In contrast to previous reports[11,14,16] one chloroxyanion byproduct that was not measured in either cantaloupe or tomatoes after $^{36}ClO_2$ (g) treatment was chlorite[18,19] (ClO_2^-). In fact, ^{36}Cl-labeled chlorite ion specifically fortified into tomatoes was quantitatively transformed to chloride and chlorate ions.[18]

Collectively, efficacy and chemical residue data suggest that the use of chlorine dioxide gas could be a highly effective, yet safe, tool for pathogen or rot organism reduction on vegetable matter. However, chemical residues of ClO_2-treated vegetable matter have not been assessed in experiments other than laboratory-scale, single exposure experiments using radiolabeled tracer materials. The purpose of this study, therefore, was to determine the magnitude of chlorate and perchlorate residues on kg-scale quantities of tomatoes and cantaloupe after sanitation with a slow-release chlorine dioxide formulation.

MATERIALS AND METHODS

Chlorine Dioxide Generation. Chlorine dioxide gas [ClO_2; CAS 10049−04−04] generation was effected using a two-part dry media system (ICA TriNova; Atlanta, GA) consisting of a zeolite carrier impregnated with sodium chlorite (Dry Media A) and an acid activator ($FeCl_3$; Part B) in a proprietary formulation. After parts A and B are mixed, chlorine dioxide gas is released in a predictable and repeatable manner.[13,20,21]

Tomato Experiments. *Containers and Accessories.* Polyethylene food storage tubs (46 × 66 × 38 cm, W × D × H; 83 L; Cambro, Huntington Beach, CA) and lids were prepared to accommodate individual flats of tomatoes, two 13 cm fans (O2Cool; Chicago, IL), a remote humidity/temperature detector (no. 14-649-84; Fisher Scientific, Pittsburgh, PA), and the gas generating media. Gas sampling ports (12 mm hole approximately 12 cm below the tub rim) were added and sealed with a butyl stopper (no. 73828A-21; Kimble Chase, Vineland, NJ) and silicone sealant. Just prior to the initiation of each experiment, container lids were lined with a thick bead of 100% silicone rubber sealant (no. 8648; DAP, Baltimore, MD).

Tomato Treatment. Ripened Beefsteak tomatoes (approximately 300 g each; DiCiocco Farms, Ontario Canada) were stored in a walk-in cooler (4−5 °C) until use. Tomatoes were removed from the cooler 1 to 1.5 h prior to the initiation an experiment and weighed to the nearest g (Table 1). Two fans were placed on the floor of each chamber and were turned on; the temperature/humidity probe was placed into the chamber. Flats containing tomatoes (n = 22 per experiment; approximately 6.6 kg total) were placed approximately 15 cm above the floor of the chamber, above the fans, on polyethylene racks. Treatments were initiated by mixing 45 g each of ICA TriNova (Newnan, GA) dry media parts A and B within a Tyvek sachet, agitating the sachet by hand to facilitate mixing, and placing the sealed sachet into the treatment chamber, but not onto the tomatoes. Sufficient media was provided to generate a target of 50 mg of chlorine dioxide per kg of tomato during a 2 h treatment period. Lids were sealed securely on each reaction chamber. Temperature and percentage relative humidity were recorded at 0, 30, 60, 90, and 120 min by reading values directly from the temperature/humidity meter. Chamber gases (5−10 mL) were removed from gas sampling ports at 0, 5, 10, 20, 30, 45, 60, 90, and 120 min using a 10 mL gastight syringe (SGE Analytical; 008960) equipped with a 19-ga syringe needle and assayed immediately for ClO_2 gas as described below.

A single chlorine dioxide treatment chamber and a single control chamber were run each d for 3 consecutive d. A third set of chambers was set up to monitor chlorine dioxide production in the absence of tomatoes. All chlorine dioxide treatments were protected from light by turning the laboratory lights off; light intensity during the sanitation process was 4−5 lx.

At the termination of the 2-h sanitation period, lids were removed from treatment tanks, tomato flats were removed, and sachets containing the ClO_2 generating media were discarded. Triplicate sets

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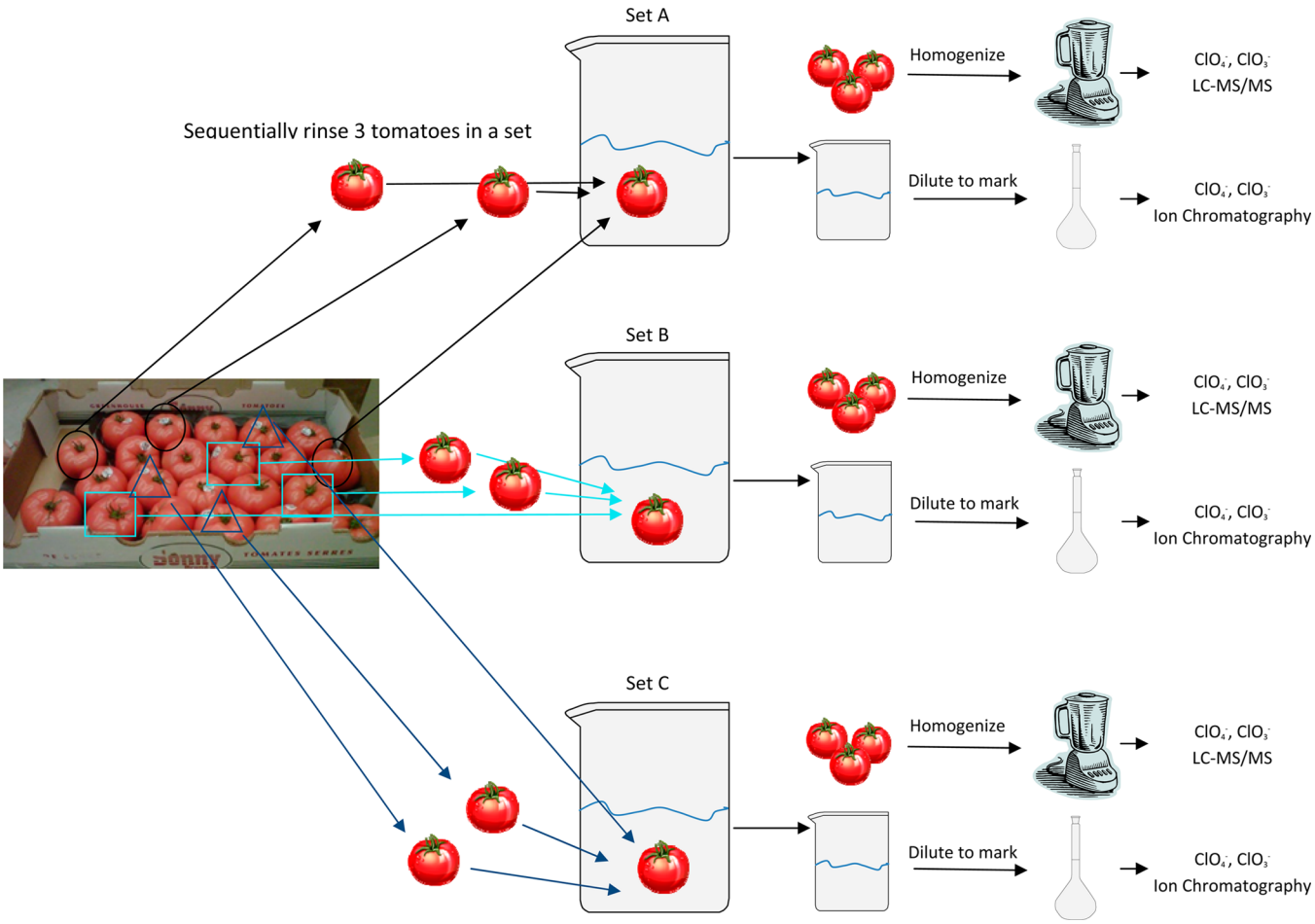

Figure 1. Schematic showing the handling of within-day tomato subsamples (Sets A, B, and C), the within set sequential rinsing of individual tomatoes, and processing of tomato sets and water rinses.

of 3 tomatoes each were removed from each flat for further processing (Figure 1). Tomato sets were weighed, and individual tomatoes within each set were sequentially rinsed in 400 mL of water (>18 MΩ cm) contained within a respective 1-L beaker (Figure 1). Rinse water for each tomato set was transferred to a 500 mL volumetric flask, diluted to the mark with purified water (>18 MΩ cm), and mixed thoroughly by inversion. Aliquots (50 mL) of each rinse fraction were placed into labeled containers and frozen (−20 °C or less) until analyses. Tomato rinses were analyzed for chlorate and perchlorate as described below.

Tomato Processing. Tomato sets were pureed in a food processor. Four 50 mL portions of the puree from each set were placed into 50 mL tubes, capped, and frozen (−20 °C or less) until analysis. Tomatoes were analyzed for perchlorate and chlorate content as described below.

Cantaloupe Experiments. *Containers and Accessories.* Polyethylene storage tubs; Rubbermaid Roughneck #3AO5; 85 × 41 × 43 cm, L × W × H; 208 L) and lids were each prepared to accommodate two cartons of cantaloupe (12 cantaloupe per carton), two 13 cm fans (O2Cool; Chicago, IL), a remote humidity/temperature detector and a Tyvek sachet as described for tomatoes. Gas sampling ports, butyl stopper seals, and silicone rubber sealant were added to containers as described for the tomato experiments.

Cantaloupe Treatment. Twenty-four cantaloupe (12-Count; Del Monte #4050, Costa Rica), equally distributed in two cardboard crates, were weighed and placed into tubs. Chlorine dioxide treatment was initiated by mixing 314 to 328 g each of dry media parts A and B within a Tyvek sachet, agitating the sachet by hand to facilitate mixing, and placing the sealed sachet into the treatment chamber (Figure 2). Sufficient media was provided to generate a target of 100 mg of chlorine dioxide per kg of cantaloupe during a 6-h treatment period. A third set of chambers (positive controls) was set up to monitor chlorine dioxide production in the absence of cantaloupe. All treatment chambers were protected from exposure to light by turning the laboratory lights off; light intensity during the sanitation process was 4−5 lx. Temperature and percentage relative humidity were recorded at 0, 60, 120, 240, and 360 min and chlorine dioxide concentration in 3 to 10 mL of chamber gas was measured at 0, 15, 30, 45, 60, 90, 120, 240, and 360 min as described for the tomato experiments. The experiment was replicated 3 times on each of 3 separate days; individual cantaloupes within a replicate were considered aliquots for which within-day residue means were calculated. Control cantaloupe treatments, which were not exposed to chlorine dioxide, consisted of a single crate of 12 melons.

Cantaloupe Processing. At the termination of the 6-h treatments, lids were removed from treatment tanks and the sachets containing ClO_2 generating media were discarded. Six cantaloupe (25% of total) were removed from treatment tanks and three cantaloupe (25% of total) were removed from control tanks with equal sampling from top and bottom layers of cantaloupe. Selected cantaloupes were weighed, bisected with a sharp knife, and the seed bed from both halves of each melon were removed, transferred to labeled containers, and weighed. The edible flesh was removed from one-half of each melon using a spoon and placed directly into a blender (Cuisinart CBT-500 or BFP-10CH, Stamford CT; or Oster BCCG08, Boca Raton, FL) where it was homogenized. Quintuplicate aliquots (~40 mL) of edible flesh homogenate from each melon were transferred to 50 mL polypropylene tubes (Sarstedt; Newton, NC; no. 62.554.002) and frozen (−20 °C). The remaining half of each melon was cut into strips and the rind with edible flesh was homogenized together.

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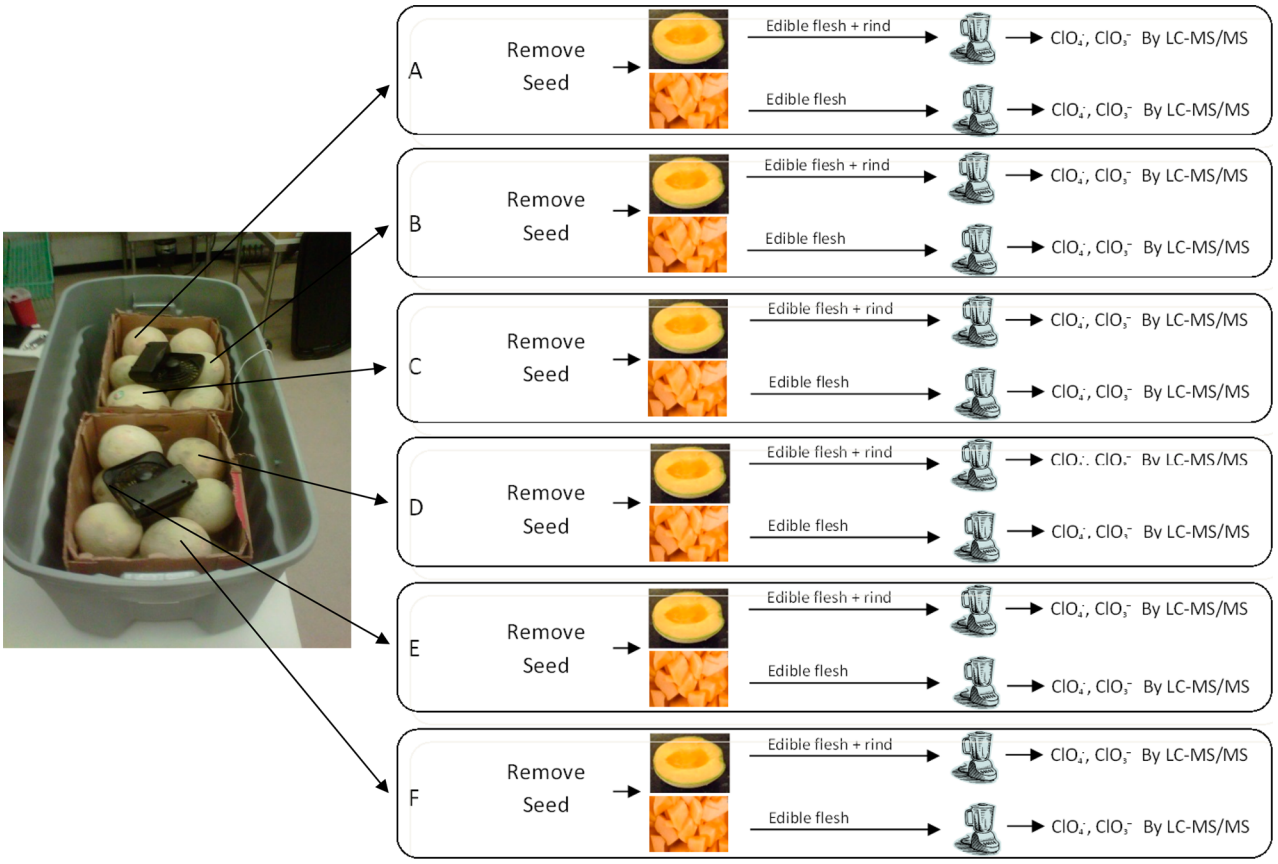

Figure 2. Daily processing of fumigated cantaloupe after ClO_2 sanitation. Of 24 cantaloupe (12 per crate; 2 crates) within an exposure tank, 3 melons were randomly selected from each crate for further processing and analysis. Equal numbers of cantaloupe were selected from the bottom and top melon layers. Cantaloupe were partitioned into edible flesh, edible flesh with rind, and seed bed fractions. Chlorate and perchlorate analyses were conducted on tank rinse, edible flesh, and edible flesh with rind fractions. Control tanks contained 12 melons, from which 3 were selected for further processing.

Quintuplicate aliquots (~40 mL) of rind and edible flesh homogenate were transferred to 50 mL polypropylene tubes and frozen.

Tank Rinse. Treatment tubs and lids were thoroughly rinsed by spraying all surfaces with nanopure (>18 MΩ cm) water from a spray container. Sequential rinses of a given tub were transferred, and pooled, into 2-L volumetric flasks. Upon completion of tank rinses, volumetric flasks were diluted to the mark, mixed thoroughly, and quintuplicate 40−45 mL aliquots were transferred into 50 mL polypropylene tubes and frozen (<−20 °C).

Chemical Analyses. *Chlorine Dioxide Assay.* A chlorine dioxide standard solution was prepared by reacting sodium chlorite with sulfuric acid as described by Ray et al.[22] Chlorine dioxide was trapped in ice-cold water after passing through a sodium chlorite column to remove Cl_2. The concentration of chlorine dioxide in the stock solution was determined by UV absorption (360 nm) of 1:10, 1:20, 1:50, and 1:100 dilutions of the stock solution in water. A molar absorption coefficient of 1225 M^{-1} cm^{-1} reported by Emmert et al.[23] was used to calculate chlorine dioxide concentration according to Beer's Law. The stock solution (0.738 ± 0.022 mg/mL) was stored sealed within a low actinic glass reservoir at 4 °C.

Chlorine dioxide concentrations in treatment chambers were measured using a Rhodamine-B based spectrophotometric assay as described by Xin and Jinyu.[24] Briefly, a standard curve containing concentrations of 0.1, 0.25, 0.5, 1.0, and 1.5 mg/L of chlorine dioxide was prepared by combining 2 mL of 10 mg/L rhodamine B, 2 mL of 1 M ammonia buffer (pH 10), and 2 mL of the an appropriate ClO_2 dilution into 25 mL volumetric flasks and diluting to the mark with purified water. After mixing, the absorbance of each vial was read at 553 nm.

Aliquots (5 to 10 mL) of gaseous chlorine dioxide were removed from containers at the indicated sampling times, bubbled immediately through respective mixtures of 1 mL of rhodamine B (10 mg L^{-1}), 1 mL of 1 M NH_3−NH_4Cl buffer (pH 10), and 10.5 mL nanopure water contained within individual 20 mL glass vials; absorbance (553 nm) was then measured using a Shimadzu (Kyoto, Japan) UV-1601 spectrophotometer. For the cantaloupe experiments, the total volume of the rhodamine B trapping solution was 25 mL, but with reagents combined in the same proportion. Concentrations of chlorine dioxide were determined using a standard curve prepared from standardized chlorine dioxide. Limits of quantitation for the Rhodamine-B chlorine dioxide assay were defined as the mean background concentration of chlorine dioxide in untreated tomato and cantaloupe tanks times 3 standard deviations of the mean.

Chlorate and Perchlorate in Rinse Waters. Perchlorate analyses of tomato rinses and cantaloupe tank rinse waters were conducted using a Thermo-Fisher ICS-2100 ion chromatograph using the framework outlined in EPA method 314.0.[25] Sample aliquots (1 mL) were injected onto a Dionex AS16 column (4 mm × 250 mm) protected by an AG16 guard column (4 mm × 50 mm). An isocratic mobile phase of 50 mM KOH, prepared using a Thermo-Fisher eluent generator, with a flow rate of 1.5 mL/min was used to elute perchlorate from the column. Perchlorate was measured using suppressed conductivity detection (Thermo-Fisher DS6) in-line with eluent-recycled (ASRS 300) suppression (186 mA). Sodium perchlorate standards (1, 5, 10, 50, 100, and 200 μg/L), prepared in nanopure water (>18 MΩ cm), were injected in replicate 1 mL aliquots onto the ion chromatograph with each sample set. Peak areas associated with perchlorate standards were regressed against perchlorate concentration using Chromeleon CHM-2 software. The least-squared regression equations were then

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used to predict the concentration of perchlorate in the experimentally obtained samples. The method detection limit (MDL) was calculated as follows:

$$\mathrm{MDL} = (t)x(S_{n-1})$$

where t is the student's t value for a 99% confidence interval (3.14 for seven replicates), and S_{n-1} is the sample standard deviation $(n-1)$ for seven replicates of the 5 ppb perchlorate standard. The MDL for perchlorate was measured contemporaneously with sample sets.

Chlorate analyses of tomato rinses and cantaloupe tank rinse waters were conducted using the same chromatograph as used for the perchlorate analyses. Standards consisting of 1, 5, 10, 50, 100, and 200 μg/L of sodium chlorate were prepared in nanopure water. Chlorate was separated from interferences on a 4 mm × 250 mm Dionex AS19 column protected by a 4 mm × 50 mm AG19 guard column with an isocratic mobile phase of 20 mM KOH flowing at 1.0 mL/min. Mobile phase was prepared using a Thermo Fisher eluent generator. Ions were detected using a DS6 conductivity detector with recycled-eluent suppression (ASRS 300; 50 mA). Sodium chlorate standards were run at the beginning and end of each sample set. Blank samples were also concurrently run with each analysis. Concentrations of chlorate in unknowns were determined using least-squared regression of peak areas of known standards. Method limits of detection were determined as described for perchlorate using the 5 ppb chlorate standard.

Perchlorate Residues in Tomato and Cantaloupe. Perchlorate in tomato puree was analyzed using the Krynitsky et al.[26] method employed by the US-Food and Drug Administration during their 2004−2005 survey of perchlorate in food.[27] Briefly, 10-g aliquots of thawed tomato puree were weighed into 50 mL conical tubes in duplicate. Sample sets included fortified control (30 ng/g) tomatoes, chlorine dioxide fumigated tomatoes, and control tomatoes. Fortified control tomatoes were obtained from a local source and were previously determined to be perchlorate free. All samples were fortified with 30 ng/g of ^{18}O-labeled perchlorate internal standard (Icon Services, Inc.; Summit, NJ). Each tube was diluted with 20 mL of 1% acetic acid and mixed at high speed for 2 min on a Rotamix (ATR; Laurel, MD) and subsequently centrifuged at 30 600 × g for 15 min on a Sorvall centrifuge at 4 °C. Supernatants were decanted into 50 mL tubes and placed on ice. Aliquots (6.5 mL) of supernatant were subsequently loaded onto preconditioned (6 mL acetonitrile followed by 6 mL of 1% acetic acid) ENVI-Carb (500 mg, 6 cc) solid phase extraction tubes. Perchlorate was not retained on the SPE tubes and was collected into tubes with the liquid portion of the tomato extract. Aliquots (1 mL) were subsequently filtered (0.2 μm PTFE filters) into 2 mL autosampler vials and 20 μL aliquots were analyzed by LC−MS/MS as described below.

Perchlorate residues were quantified in cantaloupe edible flesh and in rind with edible flesh exactly as described for tomatoes except that control cantaloupe was fortified with 150 ng/g of perchlorate; cantaloupe edible flesh was centrifuged at 30 600 × g and rind with edible flesh was centrifuged at 48 800 × g for 15 min.

Mass Spectrometry-Perchlorate. A Waters (Milford, MA) Acquity UPLC system online with a Waters triple-quadrupole mass selective detector was used to quantify perchlorate in tomato and cantaloupe edible flesh and edible flesh with rind extracts. Data were acquired, processed, and quantified using MassLynx 4.1 with QuanLynx software. Ion chromatograms were constructed for the ^{35}Cl transition m/z 99 → 83 for native perchlorate and the ^{35}Cl transition m/z 107 → 89 for ^{18}O-perchlorate. ^{37}Cl-Isotope transitions of native perchlorate were used for confirmatory purposes.[26] Sample aliquots (20 μL for tomato; 22 μL for cantaloupe) were injected from an autosampler maintained at 4 °C onto a Waters Ion-Pak Anion HR column (4.6 mm× 75 mm) maintained at 35 °C and eluted with an isocratic mobile phase of 100 mM ammonium acetate in 50% acetonitrile at a flow rate of 0.35 mL/min. Ions were detected in the negative ion mode with a capillary setting of 3.00 kV and a cone voltage of 65 V for $Cl^{18}O_4^-$ and ClO_4^-; the source and desolvation temperatures were set at 150 and 400 °C, respectively, with cone and desolvation gas flows at 50 and 800 L/h, respectively.

Calibration standards in water contained 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, and 100 ng/mL of sodium perchlorate containing 10 ng/mL of internal sodium ^{18}O-perchlorate standard. Standard curves bracketed sample sets composed of fortified blanks, test samples, and control samples. The instrument limit of quantitation was 0.5 ng/mL with the method LOQ being 1.5 ng/mL after accounting for sample mass and dilution.[26] The instrument detection limit (0.08 ng/mL for tomato experiment, 0.10 ng/mL for cantaloupe experiment) was calculated as described above using the 0.5 ng/mL standard and a t value associated with (S_{n-1}) where S is the standard deviation associated with 12 observations. Accounting for dilution and sample mass, the method LOD was 0.24 ng/mL for the tomato experiment and 0.3 ng/mL for the cantaloupe experiments.

Mass Spectrometry-Chlorate. Relative to perchlorate, tomato puree and cantaloupe edible flesh and edible flesh with rind homogenates proved to be difficult matrices for the quantitation of chlorate anions by mass spectrometry. Matrix interferences prevented the use of an ^{18}O-labeled chlorate internal standard, and the response of the ^{37}Cl-isotope transition of m/z 84.7 → 68.7 was not linear with respect to concentration in fortified samples. Therefore, quantitation of chlorate in tomato matrix was based on the ^{35}Cl-isotope transition of m/z 82.7 → 66.7 using a matrix-matched standard curve. Briefly, aliquots (10 g) of puree or homogenate were prepared for mass spectral analysis exactly as described for perchlorate except that samples were not fortified with internal standard and fortified recovery samples were spiked with 120 ng/g of sodium chlorate. A matrix-matched calibration curve, prepared in the appropriate blank sample matrix, consisted of points at 10, 20, 40, 60, 80, and 100 pg/μL. For tomatoes, the 10 pg/μL matrix-matched standard did not routinely provide a signal-to-noise ratio greater than 5, so the limit of quantitation corresponded to the 20 pg/μL matrix-matched standard with the detection limit calculated as described for perchlorate in water. A corresponding method LOQ of 60 ng/mL resulted when sample mass and dilution were accounted for; the method limit of detection corresponded to 36 ng/g. For the cantaloupe extracts, the limit of detection for the instrument, as calculated from the 10 pg/μL sodium chlorate standard was 5 ng/μL. When sample mass and dilution were accounted for, the method LOD was 15 ng/g with a corresponding LOQ of 30 ng/g.

The Acquity UPLC system equipped with a Waters triple-quadrupole mass selective detector used to quantify perchlorate was also used to quantify chlorate in tomato extracts. Ion chromatograms were constructed for the ^{35}Cl-isotope transition of chlorate ion (m/z 82.7 → 66.7). Sample aliquots (22 μL) were injected from an autosampler maintained at 4 °C onto a Waters Ion-Pak Anion HR column (4.6 mm × 75 mm) maintained at 35 °C and eluted with an isocratic mobile phase of 100 mM ammonium acetate and acetonitrile (1:1) at a flow rate of 0.35 mL/min. Ions were detected in the negative ion mode with a capillary setting of 2.6 kV and a cone voltage of 45 V for ClO_3^-; the source and desolvation temperatures were set at 150 and 400 °C, respectively, with cone and desolvation gas flows at 50 and 800 L/h, respectively.

Because chlorate concentrations of cantaloupe rind with edible flesh exceeded the highest point of the calibration curve during the initial analysis, sample dilutions were required. For those samples, dilutions (1/4 to 1/10) were made by pipetting 1 part of sample extract into the appropriate amount of 1% acetic acid to a total volume of 1200 μL. Samples were then vortexed and analyzed by LC−MS/MS as described above. For example, a 1/4 dilution was performed by adding 300 μL of sample extract to 900 μL of 1% acetic acid; a 1/10 dilution was performed by adding 120 μL of sample extract to 1080 μL of 1% acetic acid.

Statistics. Differences in overall mean reaction tank temperatures were determined by a simple one-way ANOVA after pooling all temperature measurements across time within treatments. Bonferonni's multiple comparison test was used to infer differences in treatment means after the one-way ANOVA implied significant differences in means could have occurred. Effects of treatment on perchlorate and chlorate concentrations were determined by one-way ANOVA (SigmaPlot, 12.0) with significance set at $P < 0.05$.

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RESULTS AND DISCUSSION

Table 1 shows the temperatures and relative humidity during the tomato and cantaloupe treatments. Reactions were completed at temperatures which ranged from 15.7 to 23.5 °C. The lowest temperatures occurred in chambers containing cantaloupes which had been previously refrigerated, and the highest temperatures occurred in control tomato chambers in which no fruit were added. Relative humidity tended to increase in chambers containing tomatoes and cantaloupe as a function of time, but remained fairly consistent in control tanks over the incubation periods.

Figure 3 shows the theoretical release of ClO_2 gas into treatment chambers and shows the measured concentrations of

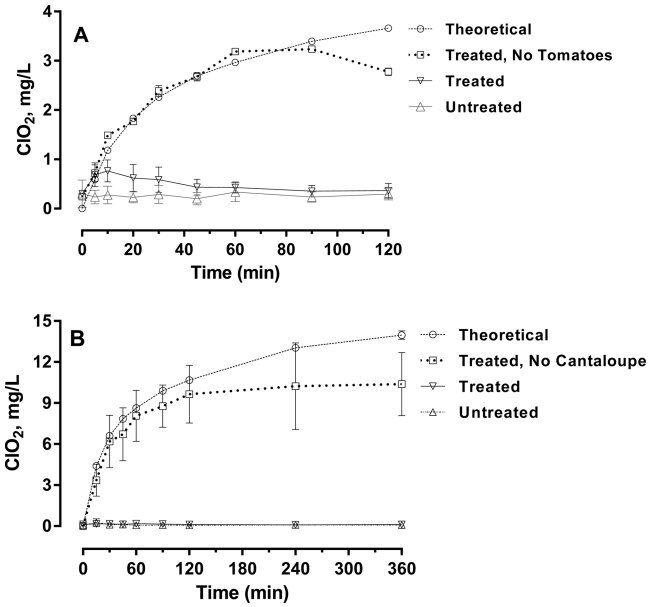

Figure 3. Mean chlorine dioxide concentrations (±standard deviations; n = 3 observations per treatment/time) in treatment tanks containing tomatoes (Panel A) or cantaloupe (Panel B). Data represent chlorine dioxide release in the absence of fruit (open squares), chlorine dioxide with fruit present (downward triangles), and in control tanks with no chlorine dioxide (upward triangles). Also shown is the theoretical release (open circles) of chlorine dioxide.

ClO_2 in control (no fruit) and fruit-laden chambers during sanitation. For both the tomato and cantaloupe experiments, chambers with an intact ClO_2 generating system, but without fruit, had ClO_2 concentrations that approximated theoretical values calculated based on ClO_2 release rates (provided by ICA TriNova; Newnan, GA) and chamber volumes. In the empty tomato chambers (Figure 3, panel A), gas concentrations approached 3 mg/L, whereas in the empty cantaloupe chambers (Figure 3, panel B) ClO_2 concentrations reached approximately 9 mg/L at 2 h and then remained relatively constant. In contrast, ClO_2 concentrations in treated tanks containing cantaloupe or tomatoes never approached theoretical levels, especially for cantaloupe (Figure 3, panel B). These data are highly consistent with studies employing $^{36}ClO_2$ that demonstrated the capacity of tomatoes and cantaloupe to adsorb ClO_2.[18,19] For instance, essentially all (>99.99%) of the radioactive residue associated with cantaloupe was on the inedible rind fraction of the melon.[18] In tomatoes, radioactive residues were highly concentrated in porous surfaces such as the stem scar where water exchange may take place.[18] Arango et al.[28] also demonstrated the capacity of produce to serve as a chlorine dioxide sink, establishing that strawberries consumed 15% of a 5 mg/L chlorine dioxide treatment within 7 min, and that chlorine dioxide absorption is a rapid, first-order process.

Since ClO_2 did not accumulate in tanks containing tomatoes or cantaloupe, a reasonable question is whether sufficient concentrations of gas for efficacy against pathogens and(or) rot organisms would be present when slow-release formulations are used. Previous researchers, however, have used similar slow release formulations to consistently reduce (>3 log units) *Salmonella*, *E. coli,* and(or) *Listeria* on the surfaces of apples, blueberries cabbage, carrots, lettuce, peaches, and tomatoes.[13,21,29−32] In addition, slow-release materials have also demonstrated efficacy against *Salmonella* on porous surfaces such as stem scars and surface wounds.[33] Further, rot or spoilage organisms including *Pseudomonas aeruginosa* and *Alicyclobacillus acidoterrestris* (spores) were reduced 5-log units or more on potatoes and apples, respectively, using slow-release ClO_2 materials.[34,35] Thus, the concentration of ClO_2 gas may not be as important as the total mass of gas delivered to fruit surfaces colonized by pathogen, rot, or spoilage organisms. An additional safety benefit with the slow release and rapid absorption of chlorine dioxide is that gas does not accumulate during sanitation. Because chlorine dioxide is a hazardous gas with implications for occupational exposures, the practical implications of nonaccumulating gas concentrations for sanitation facility infrastructure requirements and worker safety are obvious.

To be sure, gas concentrations in the empty tanks were sufficient to equal or surpass target concentrations of ClO_2 previously demonstrated to reduce *Escherichia coli* 0157:H7, *Salmonella,* and(or) *Listeria monocytogenes* on tomatoes[13,33,36] and cantaloupe[37] by 3.5 to 5 log units. Gas concentrations in the cantaloupe experiment did not reach the 10 mg/L concentration used by Trinetta et al.[15] to demonstrate the very rapid (180 s) inactivation of pathogens on tomatoes, cantaloupe, and strawberries.

Studies published after tomato or cantaloupe treatment with $^{36}ClO_2$ have established that chlorate (ClO_3^-) and chloride (Cl^-) are the major residues formed during ClO_2 sanitation of tomatoes and cantaloupe;[18,19] formation of perchlorate (ClO_4^-) may occur under sanitation conditions[18] of high gas concentration and exposure to light. Because perchlorate and chlorate are the stable residues formed, we investigated their presence in rinses of tomatoes and reaction chambers and on tomatoes and cantaloupe. Table 2 shows perchlorate and chlorate residues present in tomato rinsewater and in cantaloupe tank rinsewater from this study. Detectable (>1.3 ng/mL) perchlorate was not present in any of the tomato rinse fractions, nor was perchlorate present in the tank rinse samples of the cantaloupe experiments. The absence of perchlorate in rinses of chambers containing either tomatoes or cantaloupe is not surprising since the vegetable matter acted as chlorine dioxide sinks (Figure 3) which prevented the accumulation of chlorine dioxide gas. The fact that chlorine dioxide did not accumulate in reaction chambers likely contributed to the fact that no perchlorate was detected in rinse fractions. However, a more important factor was the absence of light during the sanitation process. For example, the absence of perchlorate in control tank rinses of the cantaloupe experiment (where chlorine dioxide did accumulate) demonstrated that in the absence of a light catalyst, the formation of perchlorate residues was prevented completely. The light-catalyzed degradation[38]

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Table 2. Concentrations of Chlorate and Perchlorate in Water Rinses of Tomatoes, And in Water Rinses of Sanitation Chambers after Treatment of Cantaloupe with Chlorine Dioxide Gas[a]

treatment	chlorate (ng/mL)	perchlorate (ng/mL)
tomatoes		
control, no ClO_2	<LOD[b]	<LOD[b]
treated, ClO_2 (+) tomatoes	6.8 ± 1.9[c]	<LOD
cantaloupe		
control, no ClO_2	<LOD[d]	<LOD[d]
treated, ClO_2 (+) cantaloupe	(4.3 ± 1.6)[e]	<LOD[d]
treated, ClO_2 (−) cantaloupe	82.1 ± 31.9	<LOD[d]

[a]Values represent means ± standard deviations of three sanitation experiments each with tomatoes and cantaloupe. [b]LOD, limit of detection for chlorate and perchlorate in tomato rinsewater was 1 and 1.3 ng/mL, respectively. [c]Two of three replicates had residues above the limit of quantitation (5.0 ng/mL); a single replicate had residues at the limit of quantitation. The mean was calculated by including the values of the single replicate having a chlorate concentration at the LOQ. [d]LOD, limit of detection for chlorate and perchlorate in cantaloupe tank rinsewater was 3.3 and 1.7 ng/mL, respectively. [e]Two of three replicates had residues below the limit of quantitation (5.0 ng/mL) but above the limit of detection (3.3 ng/mL), while one replicate had residues above the limit of quantitation. The mean was calculated by including the nominal values of the replicates having concentrations below the LOQ.

and oxidative formation of chlorate and(or) perchlorate from high concentrations of chlorine dioxide in gaseous[18,39,40] or aqueous phases[41−43] has been established in the absence of a chlorine dioxide sink as has the relative stability of gaseous chlorine dioxide gas when protected from light.[38] Previous radio labeled studies[18] surprisingly showed that even low gas concentrations in the presence of a sink could participate in light-catalyzed degradation, as small amounts of perchlorate and greater amounts of chlorate were detected when the experimental systems were exposed to light. Results from this study confirm the expectations from previous work that in the presence of a chlorine dioxide sink and in the absence of light, perchlorate formation from chlorine dioxide is nil.

Unlike perchlorate residues, chlorate residues were present in tomato rinsewater and cantaloupe tank rinsewater, albeit in low quantities (Table 2). As expected, chlorate was not present in rinses of negative controls, neither being in tomatoes rinses or tank rinses not exposed to chlorine dioxide gas. After chlorine dioxide treatment in the presence of fruit, however, low concentrations of chlorate were present in tomato rinsewater (6.8 ± 1.9 ng/mL) and in cantaloupe tank rinsewater (~4.3 ± 1.6 ng/mL). Chlorate rinsed from tomato surfaces or in rinses of cantaloupe tank chambers were just above (tomato rinses) and just below (cantaloupe tank rinses) the assay limit of quantitation (5 ng/mL). Rinse water from tanks containing ClO_2, but no cantaloupe, contained 82.1 ng/mL of chlorate. In the absence of a ClO_2 sink (cantaloupe), small quantities of chlorate were formed from ClO_2, very likely through disproportionation.[44]

Chlorate and perchlorate residues in tomato and cantaloupe homogenates are shown in Table 3. Recoveries of chlorate from blank samples fortified at 60, 120, 180, and 300 ng/g were 71.7 ± 7.8 (n = 5), 78.0 ± 8.9 (n = 6); 84.2 ± 3.8 (n = 5), and 78.4 ± 4.0%, respectively. Due to matrix interferences, the chlorate assay had an LOQ of 60 ng/g of tomato puree, and an LOD of 36 ng/g, which was substantially greater than the LOQ of 1.5

Table 3. Chlorate and Perchlorate Residues (ng/g) in Tomato Puree, Cantaloupe Edible Flesh, And Cantaloupe Edible Flesh + Rind after Treatment with Chlorine Dioxide[a]

treatment	chlorate (ng/g)	perchlorate (ng/g)
tomato puree		
control, no ClO_2	(52.2)[b]	8.8 ± 0.6
treated, ClO_2 (+) tomatoes	(45.1)[b]	9.3 ± 0.2
cantaloupe edible flesh		
control, no ClO_2	<LOD[c]	<LOD[d]
treated, ClO_2 (+) cantaloupe	<LOD[c]	<LOD[d]
cantaloupe edible flesh with rind		
control, no ClO_2	<LOD[c]	2.2 ± 0.2
treated, ClO_2 (+) cantaloupe	1319 ± 247	1.9 ± 0.3

[a]Data are means ± standard deviations of 3 replicates. [b]A nominal value is shown. Two of three replicates had tomato puree values less than the limit of detection (36 ng/g). A single replicate (shown) had chlorate residues above the LOD, but below the limit of quantitation (60 ng/g). [c]Limit of detection for chlorate in cantaloupe edible flesh was 15 ng/g; the limit of quantitation was 30 ng/g. [d]Limit of detection for perchlorate in cantaloupe edible flesh was 0.3 ng/g; the limit of quantitation was 1.5 ng/g.

ng/g for the perchlorate assay. Chlorate was either absent, or was present at levels below the LOQ, in control tomatoes and cantaloupe. A single replicate of control tomato puree contained chlorate residues above the LOD. Treatment with ClO_2 did not cause quantifiable chlorate residues to be formed in tomato puree or in edible flesh of cantaloupe; the edible flesh + rind fraction of cantaloupe, however, contained chlorate residues (1,319 ± 247 ng/g). The high concentration of chlorate residue on rind (as compared to tank rinse) confirms the notion that as chlorine dioxide was being generated, the cantaloupe surface effectively functioned as an efficient chlorine dioxide sink. Similar to the preponderance of total radioactive residue measured on cantaloupe surfaces[18] after exposure to gaseous $^{36}ClO_2$, the data provides a rationale for chlorine dioxide efficacy when chlorine dioxide gas concentrations remain low: because the gas is attracted to the vegetable surface where microbes colonize, achievement of high gas concentration is not an absolute necessity.

Perchlorate residues were present in control and treated tomato puree (Table 3), but chlorine dioxide treatment did not (P = 0.28) increase perchlorate residues relative to the control tomatoes. Recovery of perchlorate fortified at 30 ng/g of tomato puree was 111.5 ± 2.7%. Measurable perchlorate levels in control tomatoes used in this study is not surprising as a US-FDA survey of 62 domestic tomato sets collected from across the United States and 8 tomato sets from Mexico (commonly consumed in the U.S.) contained an average of 13.7 ppb of perchlorate.[27] Tomatoes fumigated with chlorine dioxide in this study clearly did not contain perchlorate burdens that were different than background perchlorate levels in control tomatoes.

Perchlorate content (Table 3) of rind plus edible flesh (1.9 ± 0.3 ng/g) of fumigated cantaloupe did not differ (P = 0.20) from the perchlorate content of untreated rind plus edible flesh homogenates (2.2 ± 0.2 ng/g). Recovery of perchlorate from fortified blank matrices averaged 101.5 ± 3.4%. Previous measurements of perchlorate residues in cantaloupe have been quite variable, depending upon the source of cantaloupes. For example, Krynitsky et al.[45] measured a median concentration of 9.6 ng/g (range <2 to 18.2 ng/g; n = 11) of perchlorate in edible flesh of cantaloupe originating in the United States

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(Arizona). However, the same study showed that when the whole cantaloupe (edible flesh, rind, and seeds) was measured, median perchlorate concentrations more than doubled relative to the flesh alone (median 23.9 ng/g; range <2 to 39.3 ng/g; n = 11). A later study from the same laboratory[26] showed that "edible portions" of cantaloupe (source unknown) with seeds contained greatly variable concentrations of residue, ranging from 2.8 to 115 ng/g perchlorate.

Collectively, results obtained from this and previous studies[18,19] are highly consistent with the known principles of chlorine dioxide chemistry and the interactions of chlorine dioxide and chlorite with reductants present in biological materials. That is, in the presence of biological reductant, chlorine dioxide may function as a five electron oxidant:[46,47]

$$ClO_2 + 1e^- \rightarrow ClO_2^- + 2e^- \rightarrow OCl^- + 2e^- \rightarrow Cl^-$$

During sanitation of tomatoes and melons, and presumably other vegetable materials, chlorine dioxide will react very rapidly with amino acids[46,48] (tryptophan < tyrosine ≪ cysteine; k from 3.2×10^4 to 1×10^7 M^{-1} S^{1-}), glutathione[46] (k 5.8×10^2 M^{-1} S^{1-}), NADH[49] (k 7.6×10^6 M^{-1} S^{1-}), nucleotides[50] (guanosine 5′-monophosphate, k 4.5×10^2 M^{-1} S^{1-}), iron[47] (k 3.9×10^3 M^{-1} S^{1-}), and a variety of phenols[51] (k from 1.4×10^3 to 1.58×10^8 M^{-1} S^{1-}) to form chlorite ion.[46,51,52] Rate constants of these magnitudes clearly explain why chlorine dioxide did not accumulate in tanks that contained tomatoes or melons.

Once formed, chlorite ion is also subject to reduction by plant-based biomaterials, albeit at somewhat slower—but still relatively fast—rates. For example, chlorite was not measured as a residue on tomatoes[18] or melons[18,19] because chlorite is subject to chemical reduction by aldoses,[53] lignin-based phenol and nonphenolic aldehydes[52] (k 0.6 M^{-1} S^{1-} to 39 M^{-1} S^{1-}), cysteine[46] (k 3.4 M^{-1} S^{1-}), polysaccharides,[54] phenols,[55] proteins[55,56] and metal cations such as iron[47,57,58] (k 4.0×10^3 M^{-1} S^{1-}), all components of plant-based organic matter. Even when chlorine dioxide is used for industrial-scale bleaching of pulp wood, chlorite ion is considered a chemical intermediate[52] chloroxyanion species. The product of chlorite reduction, hypochlorite (OCl^-) is very short-lived, being rapidly reduced to chloride ion[46] given the thermodynamic stability of chloride ion (−I oxidation state) relative to hypochlorite (+I oxidation state).

The absence of chlorite residues on produce sanitized with chlorine dioxide is of considerable importance given previous reports describing chlorite as being absorbed and excreted intact in mammals[59,60] and the toxicological potential ascribed to the chlorite ion.[61] With the known propensity for chlorite to serve as an oxidizing agent (see previous discussion), it is not surprising that chlorite was not a measurable residue in chlorine dioxide (Cl^{4+}) treated tomatoes and cantaloupe. However, it is surprising that Abdel-Rahman et al.[59,60] reported chlorite's absorption and excretion in rats, especially considering data from animals dosed with sodium chlorate (Cl^{5+}). That is, the intermediate specie, chlorite (Cl^{3+}), was never present in tissues or urine from cattle,[62,63] swine,[64] broilers,[65] or rats[56] dosed with sodium ^{36}Cl-chlorate, even though its 6 electron reduction product, chloride ion (Cl^-), was always present. The methods[66] used by, and conclusions[59,60] of, Abdel Rahmen et al., who reported that chlorite is a stable residue in rats dosed with chlorine dioxide, chlorite, and chlorate have been refuted.[56] Instability of chlorite in biomatrices has been further demonstrated; for example 17.3 μg/mL of chlorite had a half-life of only 4.5 min in bovine ruminal fluid[67] and was a detectable, but transitory, metabolite of ^{36}Cl-chlorate in pure cultures of *E. coli*.[68] In the latter study, chlorite was only measurable by directly injecting culture fluid, without pretreatment, onto an ion chromatograph equipped with a radiochemical detector. Finally, the instability of chlorite in seemingly inactive or marginally reducing matrices such as surface, ground, and even tap waters has led the US Environmental Protection Agency to recommend that precautions (the addition of preservatives, protection from light, and refrigeration) be taken at sampling to ensure accurate analytical results during water analysis.[69]

Given the apparent instability of chlorite ion, a reasonable question is why chlorite has been reported as a stable residue in chlorine dioxide treated rats[59,60] and produce.[11,14,16,17] In the case of chlorine dioxide treated rats, Hakk et al.,[56] has provided convincing evidence that the differential precipitation and solubility methods employed[66] were inadequate to speciate and quantify chlorite, chloride, and chlorate. The amperometeric method used to quantify residues on produce, cited by Han et al.,[11] Netramai,[16] and Saschower[17] (APHA method 4500-ClO2 C-Amperometric Method I), indirectly measures chlorite, and measures chlorate by difference.[70] The method is no longer recommended by the US EPA because of poor selectivity and sensitivity as described in some detail by Hoehn et al.[70] Ion chromatographic methods used by Tsai et al.[71] and Trinetta et al.[14] for measurements in produce are quantitative and may also be specific depending upon the matrix. Tsai et al.[71] did not measure detectable (LOD 0.1 mg/kg) residues of chlorite on potatoes, but Trinetta et al.[14] documented nondetectable chlorite in rinsewater of tomatoes (LOD stated to be 0.01 mg/L) to over 1200 mg/kg (1.2 parts per thousand) of chlorite residues on alfalfa sprouts. Because Trinetta et al.[14] state that chlorite quantitation was by ion chromatography with UV detection (λ_{max} not provided), and because Trinetta et al. state that alfalfa sprouts were visibly damaged subsequent to chlorine dioxide treatment, it is possible that UV-absorbing interferences could have been measured in alfalfa sprout rinses, especially since the untreated controls would not have such damage. Alternatively, chlorite might accumulate and have sufficient stability for measurement in watery, nonacidic plants like alfalfa sprouts or lettuce. Although we did not formally assay for chlorite in tank rinses of cantaloupe or in tomato rinsewater, we did look for the appearance of chlorite in ion chromatograms of rinse waters and found no evidence for its presence (see supplementary chromatogram, Figure S1).

Results from this study suggest that under the proper conditions, slow-release chlorine dioxide gas formulations could be used to sanitize tomatoes or cantaloupes with minimal deposition of perchlorate and chlorate residues on edible plant fractions. The data suggest that slow-release chlorine dioxide sanitation could be extended to other crop groups with minimal impact on food quality due to the presence of chloroxyanion residues.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b04153.

Representative ion chromatograms showing the apparent absence of chlorite in tomato rinse waters (PDF)

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Notes

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Commercial Labels and Instructions

FruitGard®

FruitGard®

Precursor for Chlorine Dioxide (ClO_2)

For use in potato storage facilities to fumigate against non-pathogenic spoilage organisms, such as yeasts and molds.

ACTIVE INGREDIENT:	
Sodium Chlorite	3.2%
OTHER INGREDIENTS:	96.8%
TOTAL	100.0%

KEEP OUT OF REACH OF CHILDREN
CAUTION

See side/back panel for Precautionary Statements

EPA REG NO. 79814-5 EPA EST. NO. 79814-GA-001

Product protected by US Patents 5,853,689 & 6,174,508

ICA TriNova, LLC
1 Beavers St., Suite B Newnan, GA 30263 USA
770.683.9181 ***www.icatrinova.com***

KEEP CONTAINER SEALED WHEN NOT IN USE

— KEEP DRY —

WARRANTY CONDITIONS OF SALE

OUR RECOMMENDATIONS FOR USE of this product are based upon tests believed reliable. Follow directions carefully. Buyer assumes all risks of use, storage and handling of this material not in strict accordance and directions given herewith. In no case shall ICA TriNova, LLC or the seller be liable for consequential, special or indirect damages resulting from the use or handling of this product when use and/or handling is not in strict accordance with directions given herewith. The foregoing is a condition of sale by ICA TriNova, LLC and is accepted by the buyer.

FruitGard®

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION: Harmful if inhaled. Causes moderate eye irritation. Avoid contact with eyes, skin, or clothing. Avoid breathing vapors. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse. Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals.

FIRST AID

If Inhaled	• Move person to fresh air. • If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. • Call a poison control center or doctor for further treatment advice.
If On Skin	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-20 minutes. • Call a poison control center or doctor for treatment advice.
If In Eyes	• Hold eye open and rinse slowly and gently with water for 15-20 minutes. • Remove contact lenses, if present, after the first 5 minutes, and then continue rinsing. • Call a poison control center or doctor for treatment advice.

Have the Product container or label with you when calling a poison control center or doctor, or going for treatment.

You may also contact 1-800-424-9300 for emergency medical treatment information.

ENVIRONMENTAL HAZARDS
This product is toxic to fish, aquatic invertebrates, oysters, and shrimp.

PHYSICAL OR CHEMICAL HAZARDS
DO NOT mix with acids or other chemical except as provided for in the "Directions for Use." Mixing with acids or other chemicals may cause evolution of chlorine dioxide gas, which may be poisonous and explosive.

NOTE: Acid activation is intended to increase the release rate of chlorine dioxide from the granules. DO NOT combine or mix acidifiers and FruitGardTM in unapproved containers (i.e., containers that do not allow for the release of the chlorine dioxide gas) or closed containers. Trapped chlorine dioxide gas may decompose and overpressure the container or release heat and cause fire.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. FruitGard® is effective for use in controlling microbiological growth such as late blight, brown rot, and others on potatoes during storage and shipment.

The following directions explain the activation and use of these granules for treating potatoes. FruitGard® is designed to release chlorine dioxide (ClO_2) gas. Treatment **MUST** take place in a suitable enclosed space. Two such treatment sites are Storage Rooms and Shipping Containers. Personnel **MUST** vacate the treatment space during the fumigation process until chlorine dioxide levels are at or below the OSHA 0.1 ppm TWA level.

Prior to application on potatoes, this product must be activated. Acid activation is intended to increase the release rate of chlorine dioxide from the FruitGard®. Activation may be accomplished by adding liquid or solid acid activators. Activate FruitGard® material only at the point of application.

ACTIVATE IN A WELL-VENTILATED AREA. AVOID BREATHING FUMES.

DO NOT combine or mix acidifiers and FruitGard® in unapproved or non-vented containers. Trapped chlorine dioxide gas may decompose and overpressure the container or release heat and cause fire.

The amount of FruitGard® required for a given weight of potatoes can be calculated as follows:

Total wt of Potatoes	Amount FruitGard® Required
1 kg	1 gm
1 metric ton (1,000 kg)	1 kg
1 cwt (100 lbs)	1.6 oz
1 U.S. ton (2,000 lbs)	32 oz (2 lbs)

Treatment Procedure:

1. Place the required amount of FruitGard® into a suitable modified reactor. A modified reactor can be the breathable sachets provided with the FruitGard®, or a plastic container (Clamshell, box, pail, *etc*.) with a porous cover (such as Tyvek®) that allows for the release of ClO_2 gas. For very large quantities, use of multiple reactors is recommended.
2. Add the recommended amount of acid activator material to the modified reactor containing the FruitGard® as shown below:
   a) Liquid food grade acid:
      i. Add 1 once of 50 wt% citric acid solution per 1,000 gms (2.2 lbs) of FruitGard®, or
      ii. Add ½ once of 75 wt% phosphoric acid solution per 1,000 gms (2.2 lbs) of FruitGard®
   b) Solid Acid Impregnate: Mix equal amounts of FruitGard® and the solid acid impregnate material (*e.g*., Z- Series™ ZF or ZPA)
3. Mix the materials by shaking or stirring gently. FruitGard™ will become active once mixed and begin releasing chlorine dioxide gas.
4. Immediately place reactor vessel / modified reactor in the Storage Container holding the potatoes, preferably on top of the potatoes to be treated. Close the Storage Container.
5. Allow gas to freely migrate across the potatoes' surface for a minimum of 6 hours.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

STORAGE: Store in a cool (preferably <75ºF), dry, well-ventilated area away from heat or open flame. Keep container sealed when not in use. Keep dry.

PESTICIDE DISPOSAL: Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture or rinsate is a violation of Federal Law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.

CONTAINER DISPOSAL: Nonrefillable container. Do not reuse or refill this container. Triple rinse container (or equivalent) promptly after emptying Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container ¼ full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. Repeat this procedure two more times. Offer for recycling, if available.

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Safety Data Sheet

Z-Series™ FruitGard® Media

SAFETY DATA SHEET

Issue Date 04-Jun-2015 **Revision Date** 04-Jun-2015 **Version** 1

1. PRODUCT AND COMPANY IDENTIFICATION

Product identifier

Product Name ClO_2 Dry Gas Precursor

Other means of identification

Product Code FRUITGARD®; CoilCleaner™

Recommended use of the chemical and restrictions on use

Recommended Use Consult your technical service representative for specific use procedures and instructions.

Uses advised against No Information available

Details of the supplier of the safety data sheet

Emergency telephone number

Emergency Telephone Chemtrec 1-800-424-9300

2. HAZARDS IDENTIFICATION

Classification

OSHA Regulatory Status

This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Acute toxicity - Oral	Category 5
Acute toxicity - Dermal	Category 4

Label elements

Emergency Overview

Warning

Hazard statements

May be harmful if swallowed

Harmful in contact with skin

Appearance No Information available **Physical state** Granules **Odor** No Information available

Precautionary Statements - Prevention

Wear protective gloves/protective clothing/eye protection/face protection

Precautionary Statements – Response

Specific Measures (see the appropriate section of the SDS)

IF ON SKIN: Wash with plenty of soap and water

Call a POISON CENTER or doctor/physician if you feel unwell

Wash contaminated clothing before reuse

Precautionary Statements – Disposal
Dispose of contents/container to an approved waste disposal plant

Hazards not otherwise classified (HNOC)
Other Information
Unknown Acute Toxicity 0% of the mixture consists of ingredient(s) of unknown toxicity

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name	CAS No.	Weight-%	Trade Secret
Zeolite	1318-02-1	60-100	*
Water	7732-18-5	5-10	*
Sodium Chlorite	7758-19-2	1-5	*

*The exact percentage (concentration) of composition has been withheld as a trade secret.

4. FIRST AID MEASURES

First aid measures

General advice If symptoms persist, call a physician. Do not breathe dust/fume/gas/mist/vapors/spray. Do not get in eyes, on skin, or on clothing.

Skin Contact Consult a physician if necessary. Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes.

Eye contact Immediately flush with plenty of water. After initial flushing, remove any contact lenses and continue flushing for at least 15 minutes Keep eye wide open while rinsing If symptoms persist, call a physician

Inhalation Remove to fresh air. Call a physician. If breathing is irregular or stopped, administer artificial respiration. Avoid direct contact with skin. Use barrier to give mouth-to-mouth resuscitation.

Ingestion Rinse mouth. Drink plenty of water. If symptoms persist, call a physician. Do NOT induce vomiting.

Self-protection of the first aider Use personal protective equipment as required.

Most important symptoms and effects, both acute and delayed

Symptoms No Information available.

Indication of any immediate medical attention and special treatment needed

Note to physicians Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media
Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

Unsuitable extinguishing media Caution: Use of water spray when fighting fire may be inefficient.

Specific hazards arising from the chemical

Sodium Chlorite is a known oxidizer; avoid contact with organic matter. *FruitGard*® product formulations are non-flammable. Premature or accidental mixture of *FruitGard*® media or direct contact of media with acids and/or reducing agents may result in the release of gas. The gas is not flammable. In the event of accidental premature release of gas apply flooding quantities of water to quench reaction, as practical, avoid use of pressurized water.
.

Explosion data

Sensitivity to Mechanical Impact	None.
Sensitivity to Static Discharge	None.

Protective equipment and precautions for firefighters

Use protective equipment appropriate to local circumstances and the surrounding environment.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures

Personal precautions Ensure adequate ventilation, especially in confined areas. In situations where ventilation is inadequate wear appropriate air-purifying full face respirators. Use a NIOSH/MSHA acid gas approved respirator or equivalent.

Environmental precautions

Environmental precautions Prevent entry into waterways, sewers, basements or confined areas. Do not flush into surface water or sanitary sewer system.

Methods and material for containment and cleaning up

Methods for containment Prevent further leakage or spillage if safe to do so.

Methods for cleaning up **LARGE SPILLS:** Evacuate the area. Isolate hazard area and restrict access to necessary and protected personnel. Remove all sources of ignition and contain spill. Cover powder spill with plastic sheet or tarp to minimize spreading. Place contaminated material in a disposal container and thoroughly rinse spill area. Avoid material runoff into storm drains, ditches, or any pathways that lead to waterways. Never discharge into natural bodies of water. Ventilate the area thoroughly.
SMALL SPILLS: Place all contaminated material in a disposal container and thoroughly rinse spill area with water.
US Regulations (CERCLA) require reporting spills and releases to soil, water and air in excess of reportable quantities.

7. HANDLING AND STORAGE

Precautions for safe handling

Advice on safe handling Avoid contact with skin, eyes or clothing. Use personal protective equipment as required. Wash contaminated clothing before reuse. Do not breathe dust/fume/gas/mist/vapors/spray. Do not eat, drink or smoke when using this product. Except when in use, do not open individual packages to expose media components; keep bulk media containers tightly closed when not in use.

Conditions for safe storage, including any incompatibilities

Storage Conditions Keep container tightly closed in a dry and well-ventilated place. Keep out of the reach of children.

Incompatible materials Acids, reducing agents, oxidizers, combustible materials, solvents, paints and sulfur.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Control parameters

Exposure Guidelines This product, as supplied, does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

Appropriate engineering controls

Engineering Controls Showers, Eyewash stations & Ventilation systems. Or other control means to minimize airborne exposure. Otherwise, use general exhaust ventilation or other air circulation means.

Individual protection measures, such as personal protective equipment

Eye/face protection Tight sealing safety goggles.

Skin and body protection Impervious gloves are recommended, but not required.

Respiratory protection If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with current local regulations.

General Hygiene Handle in accordance with good industrial hygiene and safety practice.

9. PHYSICAL AND CHEMICAL PROPERTIES

Information on basic physical and chemical properties

Physical state Granules
Appearance Off white, light gray.
Odor Mild bleach/pool odor.

Odor threshold No Information available

Property	Values	Remarks • Method
pH	Not an Aqueous Solution	
Melting point/freezing point	No Information available	
Boiling point / boiling range	No Information available	
Flash point	None	
Evaporation rate	No Information available	
Flammability (solid, gas)	No Information available	
Flammability Limits in Air		
Upper flammability limit:	No Information available	
Lower flammability limit:	No Information available	
Vapor pressure	No Information available	
Vapor density	No Information available	
Specific Gravity	Range 80 to 110 lbs/ft3 packed	
Water solubility	1% to 10% at 25 degree centigrade.	
Solubility in other solvents	No Information available	
Partition coefficient	No Information available	
Autoignition temperature	No Information available	
Decomposition temperature	Range 250 to 300 degrees Centigrade.	
Kinematic viscosity	No Information available	
Viscosity	No Information available	

Explosive properties No Information available
Oxidizing properties No Information available

10. STABILITY AND REACTIVITY

Reactivity
Strong gas may be generated upon contact with reducing agents, acids and/or oxidizers or mishandling of packages or improper storage of packages.

Chemical stability
Stable under recommended storage conditions.

Possibility of Hazardous Reactions
None under normal processing.

Conditions to avoid
Extremes of temperature and direct sunlight.

Incompatible materials
None known based on information supplied.

Hazardous Decomposition Products
Avoid ignition sources and extended exposure to heat, moisture and ultraviolet light.

11. TOXICOLOGICAL INFORMATION

Information on likely routes of exposure

Product Information No data available

Inhalation Inhalation may cause irritation of the mucous membranes and respiratory system characterized by coughing, burning, and sneezing. Extreme overexposure may result in lung damage.

Eye contact Eye irritation may result from prolonged exposure to low levels of dust.

Skin Contact Skin irritation may result from prolonged exposure to low levels of dust.

Ingestion May be harmful if swallowed.

Chemical Name	Oral LD50	Dermal LD50	Inhalation LC50
Sodium Chlorite 7758-19-2	= 165 mg/kg (Rat)	= 107.2 mg/kg (Rabbit)	= 230 mg/m^3 (Rat) 4 h

Information on toxicological effects

Symptoms No Information available.

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Sensitization No Information available.
Germ cell mutagenicity No Information available.
Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Chemical Name	ACGIH	IARC	NTP	OSHA
Zeolite 1318-02-1	-	Group 3	-	-
Sodium Chlorite 7758-19-2	-	Group 3	-	-

IARC (International Agency for Research on Cancer)
Not classifiable as a human carcinogen

Reproductive toxicity No Information available.
STOT - single exposure No Information available.
STOT - repeated exposure No Information available.
Aspiration hazard No Information available.

Numerical measures of toxicity - Product Information

Unknown Acute Toxicity 0% of the mixture consists of ingredient(s) of unknown toxicity
The following values are calculated based on chapter 3.1 of the GHS document.

12. ECOLOGICAL INFORMATION

Ecotoxicity

0% of the mixture consists of components(s) of unknown hazards to the aquatic environment

Chemical Name	Algae/aquatic plants	Fish	Crustacea
Zeolite 1318-02-1	18: 96 h Desmodesmus subspicatus mg/L EC50	1800: 96 h Brachydanio rerio mg/L LC50 semi-static 3200 - 5600: 96 h Oryzias latipes mg/L LC50 semi-static 1800 - 3200: 96 h Poecilia reticulata mg/L LC50 semi-static	1000 - 1800: 48 h Daphnia magna mg/L EC50
Sodium Chlorite 7758-19-2	-	100 - 500: 96 h Brachydanio rerio mg/L LC50 static 100: 96 h Lepomis macrochirus mg/L LC50 static 100: 96 h Oncorhynchus mykiss mg/L LC50 static	0.026: 48 h Daphnia magna mg/L EC50 0.25 - 0.33: 48 h Daphnia magna mg/L EC50 Flow through 0.012 - 0.018: 48 h Daphnia magna mg/L EC50 Static

Persistence and degradability
No Information available.

Bioaccumulation
No Information available.

Other adverse effects No Information available

13. DISPOSAL CONSIDERATIONS

Waste treatment methods

Disposal of wastes Disposal should be in accordance with applicable regional, national and local laws and regulations.

Contaminated packaging Do not reuse container.

14. TRANSPORT INFORMATION

DOT Not regulated

15. REGULATORY INFORMATION

International Inventories
TSCA Complies
DSL/NDSL Complies
EINECS/ELINCS Complies

AICS Complies

Legend:
TSCA - United States Toxic Substances Control Act Section 8(b) Inventory
DSL/NDSL - Canadian Domestic Substances List/Non-Domestic Substances List
EINECS/ELINCS - European Inventory of Existing Chemical Substances/European List of Notified Chemical Substances
AICS - Australian Inventory of Chemical Substances

US Federal Regulations

SARA 313
Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372

SARA 311/312 Hazard Categories

Acute health hazard	No
Chronic Health Hazard	No
Fire hazard	No
Sudden release of pressure hazard	No
Reactive Hazard	No

CWA (Clean Water Act)
This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42)

CERCLA
This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material

US State Regulations

California Proposition 65
This product does not contain any Proposition 65 chemicals

U.S. State Right-to-Know Regulations

Chemical Name	New Jersey	Massachusetts	Pennsylvania
Sodium Chlorite 7758-19-2	X	X	X

U.S. EPA Label Information
EPA Pesticide Registration Number Not Applicable

16. OTHER INFORMATION

NFPA	**Health hazards 2**	**Flammability** 0	**Instability 0**	**Physical and Chemical Properties** Yes
HMIS	**Health hazards 2**	**Flammability** 0	**Physical hazards 0**	**Personal protection** N/A

Issue Date 04-Jun-2015
Revision Date 04-Jun-2015
Revision Note
No Information available

Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet

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Item B
Statement #11
Exhibit 11

Substance Research Information

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