JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

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

D. J. Smith,*[,†] W. Ernst,[‡] and J. M. Giddings[†]

[†]Biosciences Research Laboratory, Agricultural Research Service, United States Department of Agriculture, 1605 Albrecht Boulevard, Fargo, North Dakota 58102-2765, United States

[‡]ICA TriNova, LLC, 1 Beavers St., Newnan, Georgia 30263, United States

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

Received: August 28, 2014
Revised: November 10, 2014
Accepted: November 10, 2014
Published: November 19, 2014

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.

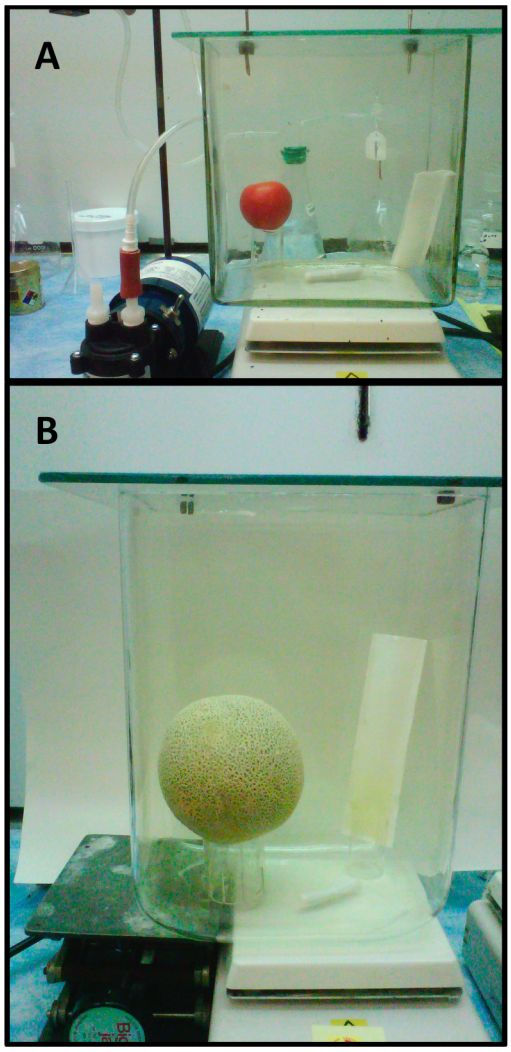

Figure 1. Experimental chambers for tomato (panel A) and cantaloupe (panel B) fumigations with ^{36}Cl-chlorine dioxide. Panel A shows a tomato experiment at the initiation of the venting process. Panel B shows a cantaloupe experiment during fumigation; ^{36}Cl-chlorine dioxide gas can be clearly seen 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

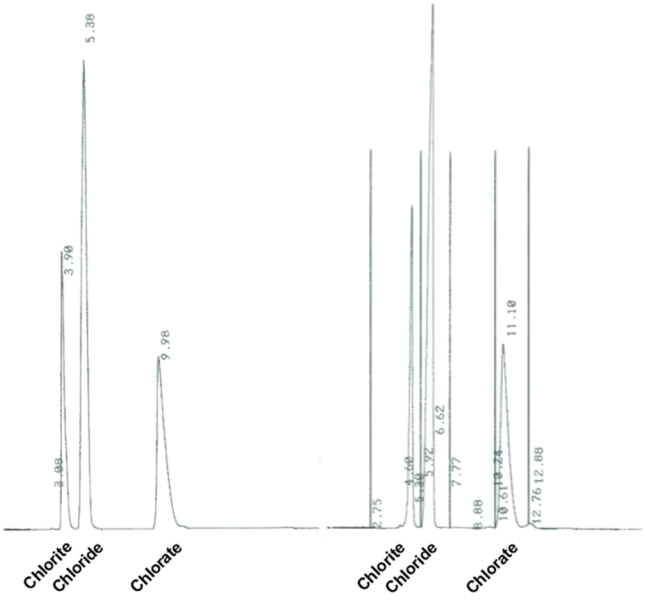

Figure 2. Ion chromatographic separation of unlabeled chlorite, chloride, and chlorate standards in water (10 μL; left panel), and an aliquot (1000 μL) of tomato rinse fortified with the standard mix (right panel). Vertical lines in the chromatogram of the tomato rinse fraction represent the regions 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).

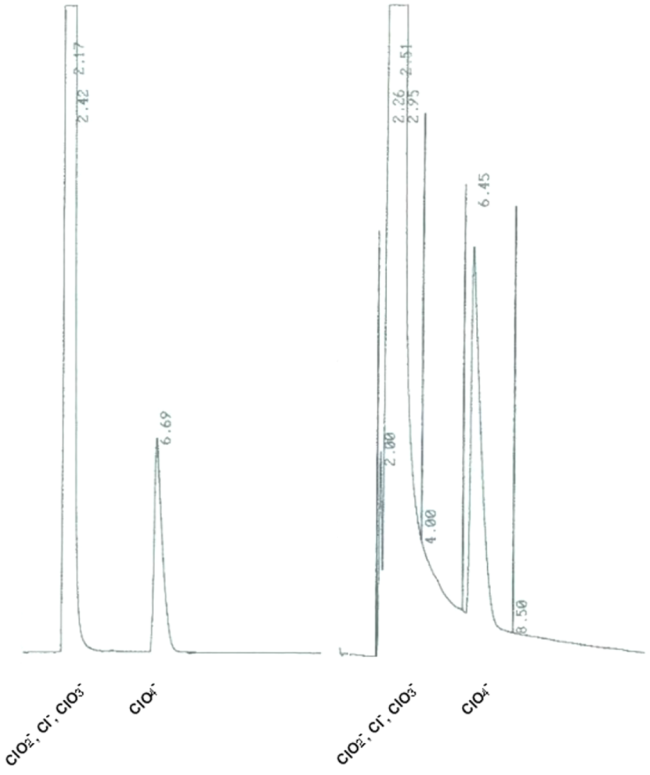

Figure 3. Ion chromatographic separation of unlabeled chlorite, chloride, chlorate, and perchlorate standards (left panel) and an aliquot of tomato serum containing incurred residue (right panel) and fortified with the standard mix. Radioactivity present as chlorite, chloride, and chlorate was not resolved 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.

AUTHOR INFORMATION

Corresponding Author

*Phone: 701-239-1238. E-mail: david.j.smith@ars.usda.gov.

Funding

This study was collaboratively funded by ICA-TriNova (Agreement No. 58-5442-1-431) and the USDA ARS (Project No. 5442-32000-014).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The use of trade, firm or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable. USDA is an equal opportunity employer.

REFERENCES

(1) United Nations, Food and Agriculture Organization. *Global Food Losses and Food Waste: Extent, Causes and Prevention*; Rome, Italy, 2011. http://www.fao.org/docrep/014/mb060e/mb060e00.pdf. Accessed 3/19/2014.

(2) Code of Federal Regulations, 40§180.41. 2013.

(3) Laio, C.-H. Bacterial soft rot. In *Microbiology of Fruits and Vegetables*; Sapers, G. M., Gorny, J. R., Yousef, A. E., Eds.; CRC Taylor and Francis: Boca Raton, FL, USA, 2006; pp 117−134.

(4) Moss, M. O. Fungi, quality and safety issues in fresh fruits and vegetables. *J. Appl. Microbiol.* **2008**, *104*, 1239−1243.

(5) Gorny, J. Microbial contamination of fresh fruits and vegetables. In *Microbiology of Fruits and Vegetables*; Sapers, G. M., Gorny, J. R., Yousef, A. E., Eds.; CRC Taylor and Francis: Boca Raton, FL, USA, 2006; pp 3−32.

(6) US EPA. *Registration of pesticides containing chlorine dioxide*; 2009. http://www.epa.gov/pesticides/factsheets/chemicals/chlorinedioxidefactsheet.htm.

(7) Trinetta, V.; Vaidya, N.; Linton, R.; Morgan, M. Evaluation of chlorine dioxide gas residues on selected food produce. *J. Food Sci.* **2010**, *76*, T11−T15.

(8) Trinetta, V.; Linton, R. H.; Morgan, M. T. Use of chlorine dioxide gas for the postharvest control of *Alternaria alternate* and *Stemphylium vesicarium* on Roma tomatoes. *J. Sci. Food Agric.* **2013**, *93*, 3330−3333.

(9) Mahovic, M. J.; Tenney, J. D.; Bartz, J. A. Applications of chlorine dioxide gas for control of bacterial soft rot in tomatoes. *Plant Dis.* **2007**, *91*, 1316−1320.

(10) Sy, K. V.; Murray, M. B.; Harrison, D.; Beuchat, L. R. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *E. coli*

O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *J. Food Prot.* **2005**, *68*, 1176−1187.
(11) Sy, K. V.; McWatters, K. H.; Beuchat, L. R. Efficacy of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, yeasts, and molds on blueberries, strawberries, and raspberries. *J. Food Prot.* **2005**, *68*, 1165−1175.
(12) Hakk, H.; Smith, D. J.; Shappell, N. W. Tissue residues, metabolism, and excretion of radiolabeled sodium chlorate ($Na[^{36}Cl]ClO_3$) in rats. *J. Agric. Food Chem.* **2007**, *55*, 2034−2042.
(13) Smith, D. J.; Anderson, R. C.; Ellig, D. A.; Larsen, G. Tissue distribution, elimination, and metabolism of dietary sodium $[^{36}Cl]$-chlorate in beef cattle. *J. Agric. Food Chem.* **2005**, *53*, 4272−4280.
(14) Smith, D. J.; Stehly, G. S.; Turberg, M. T. Procedures and practices for conducting residue studies of animal health compounds in food animals. In *Handbook of Residue Analytical Methods for Agrochemicals*; Lee, P., Ed.; John Wiley & Sons, Ltd.: 2003; Vol. *1*, pp 248−299.
(15) US EPA. *Method 314.0 Determination of perchlorate in drinking water using ion chromatography. Revision 1.0*; 1999. http://www.epa.gov/ogwdw/methods/pdfs/methods/met314.pdf (accessed 8/01/2014).
(16) Han, Y.; Floros, J. D.; Linton, R. H.; Nielsen, S. S.; Nelson, P. E. Response surface modeling for the inactivation of *Escherichia coli* O157:H7 on green peppers (*Capsicum annuum* L.) by chlorine dioxide gas treatments. *J. Food Prot.* **2001**, *64*, 1128−1133.
(17) Han, Y.; Selby, L. T.; Schultze, K. K.; Nelson, P. E.; Linton, R. H. Decontamination of strawberries using batch and continuous chlorine dioxide gas treatments. *J. Food Prot.* **2004**, *67*, 2450−2455.
(18) Mahovic, M.; Bartz, J. A.; Schneider, K. R.; Tenney, J. D. Chlorine dioxide gas from an aqueous solution: Reduction of *Salmonella* in wounds on tomato fruit and movement to sinks in a treatment chamber. *J. Food Prot.* **2009**, *72*, 952−958.
(19) Wu, V. C. H.; Rioux, A. A simple instrument-free gaseous chlorine dioxide method for microbial decontamination of potatoes during storage. *Food Microbiol.* **2010**, *27*, 179−184.
(20) White, J. F.; Taylor, M. C.; Vincent, G. P. Chemistry of chlorites. *Ind. Eng. Chem.* **1942**, *34*, 782−792.
(21) Nabar, G. M.; Ramachandran, C. R.; Shenai, V. A. Studies in sodium chlorite: Part II-The mechanism of photodecomposition of sodium chlorite. *Ind. J. Technol.* **1964**, *2*, 11−14.
(22) Vel Leitner, N. K.; De Laat, J.; Dore, M. Photodecomposition of chlorine dioxide and chlorite by U.V.-irradiation—Part I. Photoproducts. *Water Res.* **1992**, *26*, 1655−1664.
(23) Spinks, J. W. T.; Porter, J. M. Photodecomposition of chlorine dioxide. *J. Am. Chem. Soc.* **1934**, *56*, 264−270.
(24) Crawford, R. A.; Dewitt, B. J. Decomposition rate studies in the gaseous chlorine dioxide-water system. *TAPPI* **1968**, *51*, 226−230.
(25) Schack, C. J.; Pilipovich, D. Chlorine perchlorate. *Inorg. Chem.* **1970**, *9*, 1387−1390.
(26) Schell-Sorokin, A. J.; Bethune, D. S.; Lankard, J. R.; Loy, M. M. T.; Sorokin, P. P. Chlorine perchlorate: A major photolysis product of chlorine dioxide. *J. Phys. Chem.* **1971**, *86*, 4653−4655.
(27) Brickhouse, M. D.; Lalain, T.; Bartram, P. W.; Hall, M.; Hess, Z.; Mantooth, B.; Reiff, L.; Zander, Z.; Stark, D.; Humphreys, P.; Ryan, S.; Martin, B. *Effects of vapor-based decontamination systems on selected building interior materials: Chlorine dioxide*; EPA/600/R-08/054; United States Environmental Protection Agency: 2008.
(28) Cosson, H.; Ernst, W. R. Photodecompostion of chlorine dioxide and sodium chlorite in aqueous solution by irradiation with ultraviolet light. *Ind. Eng. Chem. Res.* **1994**, *33*, 1468−1475.