

Hypochlorite Treatments are not a Significant Source of Perchlorate Exposure in Lettuce

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Leafy vegetables, such as lettuce (*Lactuca sativa* L), have been identified as a potential source of perchlorate exposure to humans. Perchlorate is of concern because excessive amounts may impair thyroid function by inhibiting iodide uptake by the sodium iodide symporter. Perchlorate has been identified as an oxidation product in sodium hypochlorite. Dilute hypochlorite solutions are widely used on lettuce as a preservative and as a treatment to reduce microbial food risks. However, the potential of hypochlorite to be a source of human perchlorate exposure from lettuce had not been evaluated. Studies were conducted with lettuce collected in the San Luis Valley of southern Colorado and in the lower Colorado River Valley of southwestern Arizona to represent conditions under which hypochlorite is applied to lettuce in the field and in salad processing facilities. We used spray and dipping solutions that were dilutions of concentrated sodium hypochlorite that would contain from 12000 and 120000 $\mu\text{g/L}$ perchlorate. The perchlorate content of iceberg and romaine lettuce averaged 6.2 and 7.2 $\mu\text{g/kg}$ fw in southern Colorado and 14.0 and 56.7 $\mu\text{g/kg}$ fw in southwestern Arizona and there were no significant ($P > 0.05$) increases in the perchlorate content of lettuce due to hypochlorite treatments. Because of the relatively low concentrations of perchlorate present after dilution and the low volumes applied to lettuce, hypochlorite solutions do not appear to be a significant source of the perchlorate levels found in lettuce.

KEYWORDS: Perchlorate; lettuce; hypochlorite; sodium iodide symporter

INTRODUCTION

Environmental perchlorate exposures are of public health concern due to potential perchlorate impairment of thyroid function by inhibiting iodide uptake by the sodium iodide symporter (NIS) (1). A reference dose (RfD) of 0.7 $\mu\text{g/kg}$ has been recommended by the National Academy of Science (2). The RfD is based on a no observed adverse effect level (NOAEL) of 7 $\mu\text{g/kg}$ from a human perchlorate dosing study (3) and a 10-fold uncertainty factor to address potential sensitive subpopulations. One epidemiological study examined perchlorate exposure in Chilean women and found no changes in thyroid hormone levels despite exposure doses estimated to be higher than the RfD (4). However, another recent study found that estimated perchlorate doses below the reference dose were associated with altered thyroid hormone levels in women with

low iodine intake (5). One explanation of these different findings is that Tellez et al. (4) examined only three women with average urinary iodine $<100 \mu\text{g/L}$, while Blount et al. (5) examined 348 women with urinary iodine $<100 \mu\text{g/L}$. Increased iodine intake could decrease the ability of a given dose of perchlorate to inhibit iodide transport.

Food is a source of low level human exposure to perchlorate (6). Foods such as leafy vegetables and dairy products have been implicated in human perchlorate exposure through biomonitoring studies (7). The Colorado River is contaminated with parts-per-billion levels of perchlorate from aerospace and defense related industries. Crops irrigated with Colorado River water accumulate trace levels of perchlorate (8–10). However, work has also shown perchlorate in vegetables and milk samples collected nationally (11–13) and internationally (14, 15). A recent study showed detectable, but low, perchlorate levels in all urine specimens (estimated 95th percentile dose was 0.234 $\mu\text{g/kg-day}$) (16). While the sources were not determined from these data, these detections indicate widespread human exposure, albeit at doses below the EPA RfD. Various sources of national perchlorate exposure are not well defined, but both anthropogenic and natural sources are known (17, 18).

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Recently, commercial sodium hypochlorite has been identified as a potential source of perchlorate (19). Perchlorate is an oxidation product of hypochlorite. Perchlorate concentrations up to 8000 $\mu\text{g/L}$ have been found in commercial sodium hypochlorite products. A recent survey found perchlorate in over 90% of the hypochlorite products used in water treatment facilities (20). Interestingly, hypochlorite solutions are widely used on agricultural produce as a preservative during storage and shipping and as a treatment to reduce microbial food risks (21). Field grown lettuce is often treated with dilute hypochlorite after hand harvesting and before wrapping and boxing. In addition, lettuce processed for prepackage salads receive multiple applications of increasingly diluted hypochlorite solutions. In previous work with lettuce and other leafy vegetables (8, 13), most samples were collected directly from the field immediately prior to commercial harvest operation. The potential for hypochlorite treatment to contribute to perchlorate exposure in leafy vegetables was not evaluated. The objective of these experiments was to evaluate whether hypochlorite treatment leads to significantly higher perchlorate levels in lettuce.

MATERIALS AND METHODS

Field Application. Two field experiments were conducted in 2006–2007 to evaluate the potential for hypochlorite to increase the perchlorate content of lettuce. The first field experiment was conducted in the San Luis Valley of southern Colorado in the summer of 2006 because lettuce from this area was found to have the lowest background concentrations of perchlorate in a previous study (13). Further, this area was in production when we realized that hypochlorite was a potential source of perchlorate in lettuce. Evaluations in the field included both mature iceberg and romaine lettuce. We applied hypochlorite solutions in a manner to reproduce their normal application under field conditions.

A final concentration of 200 mg/L hypochlorite was made by diluting 3.2 mL of a 6% sodium hypochlorite solution per liter of water. The commercial sodium hypochlorite solution we used had a background perchlorate concentration of 12000 $\mu\text{g/L}$. Thus, after dilution for spraying, the nominal concentration of perchlorate of approximately 40 $\mu\text{g/L}$ was confirmed by analysis. We also simulated spray application using sodium hypochlorite solutions containing perchlorate above the upper range of reported levels. For this, we mixed another hypochlorite solution spiked with perchlorate to achieve a final concentration after dilution for spraying of 385 $\mu\text{g/L}$. This would correspond to using a concentrated sodium hypochlorite solution having an initial perchlorate concentration of 120000 $\mu\text{g/L}$. All solutions were prepared from local well water in the San Luis Valley. This water had no detectable perchlorate by IC/MS/MS. Thus, the experimental treatments included an untreated control, a hypochlorite spray solution containing 40 $\mu\text{g/L}$ ClO_4^- , and a hypochlorite solution containing 385 $\mu\text{g/L}$ ClO_4^- . For all treatments we also compared the perchlorate concentrations from lettuce with no post-harvest modification, trimming after one day of cold storage, and rinsing with water after one day of cold storage. This supplemental trimming and washing was intended to mimic the additional trimming and washing performed by retailers.

Lettuce was harvested and trimmed to market grade, turned upside down (tops down and basal stem pointed up), and sprayed to coverage using a backpack sprayer as is done commercially. Based upon application time and rate, we estimated by volumetric measurements that less than a 5 mL spray was applied to each lettuce plant. The experimental design was completely random with five replications. The samples were diced, mixed, subsampled, and shipped on dry ice to our laboratory in Yuma, Arizona. The samples were frozen until analysis.

Because of anomalous observations for the romaine data in the first field experiment, a second experiment with romaine was conducted in the lower Colorado River Valley of Arizona in the spring of 2007. Hypochlorite solutions were prepared as described above but we used 200 mg/L hyperchlorite solutions having 54 and 237 $\mu\text{g/L}$ perchlorate. This would represent dilution of 6% hypochlorite solutions having initial

perchlorate concentrations of approximately 17000 and 74000 $\mu\text{g/L}$ perchlorate. These solutions were prepared with DI water because wells in the lower Colorado River region have low levels of perchlorate from the Colorado River. For the untreated control and hypochlorite sprays, we compared no post harvest modification and trimming. The experiment was completely random with five replications. In this experiment we also took additional steps in mixing the sample more thoroughly prior to subsampling in an attempt to avoid variation that may have been associated with earlier subsample errors. The samples were transported to our laboratory and frozen until analysis.

Salad Processing. Lettuce processed for salads often receives multiple hypochlorite applications. Initial application usually occurs in the field, as demonstrated above for field packed lettuce. Subsequent applications of increasingly dilute chlorine solutions occurs in the salad processing facility. This experiment was conducted to simulate a typical sequence of sprays and dips that occur for processed lettuce. These evaluations were performed with lettuce produced in the lower Colorado River Valley of southwestern Arizona.

All solutions were made from a concentrated sodium hypochlorite solution noted above and diluted appropriately with DI water. The solutions were spiked with perchlorate to simulate using a concentrated solution with an initial perchlorate concentration of 112000 $\mu\text{g/L}$. Subsequent chlorine and perchlorate concentrations would reflect the approximate appropriate serial dilutions of this concentrated solution.

A total of nine lettuce heads collected at random from the lettuce field were shredded and stored frozen for analysis. A total of 27 heads were sprayed in the field, as described above, with a 200 mg/L hypochlorite solution having 355 $\mu\text{g/L}$ perchlorate. Nine of these heads were collected at random, shredded, and stored frozen for analysis. The remaining 18 heads were shredded and all 18 individual samples were dipped in a 60 mg/L chlorine solution having 124 $\mu\text{g/L}$ perchlorate. After drainage and drying in a cooler, nine of these subsamples were collected at random and frozen for analysis. The remaining nine subsamples were dipped into a 20 mg/L chlorine solution having a perchlorate concentration of 40 $\mu\text{g/L}$, dried in a cooler, and frozen until analysis.

Sample Processing and Extraction for Perchlorate. The frozen samples were freeze-dried on a Labconco freeze drier. Weights before and after freeze-drying were recorded. The samples were ground and stored in vials for extraction. We used an extraction procedure described previously (22) with minor modifications. Briefly, 600 mg of freeze-dried product was weighed into centrifuge tubes and 15 mL of DI water were added. The tubes were boiled for 30 min and the contents were placed in a refrigerator overnight with occasional gentle shaking. The tubes were then centrifuged for 30 min and the supernatants filtered sequentially through Kim wipes and 0.2 μm Gel-man ion membrane syringe filters. A total of 2 mL of the above extract (extract one) was slurried with 1000 mg DD6 alumina. Vials were gently agitated two or three times over a 24 h period, after which 18 mL of DI water was added to the mixture. After stirring and settling, this solution was filtered through another 0.2 μm Gel-man ion membrane syringe filter, and the resulting solution was labeled "extract 2". This sample was stored in the freezer until analysis by ion chromatography with conductivity detection (IC-CD) and ion chromatography tandem mass spectroscopy (IC/MS/MS).

Perchlorate analyses were initially performed by IC-CD using a Dionex 2500 described previously (13). However, a large number of the samples collected in these experiments were below detection by IC-CD (<20 $\mu\text{g/kg}$ fw). Further, because we judged that we needed IC/MS/MS for detection of any potential small differences caused by hypochlorite sprays, a large subset of samples were reanalyzed using IC/MS/MS. All data reported and used in statistical evaluations were analyzed by IC/MS/MS. This included only four of the five replications for the first field experiment but all the data from the second field study and the salad processing study. The IC/MS/MS method has been described in detail previously (23). Briefly, 0.5 mL of aqueous sample extract was spiked with an isotopically labeled internal standard (2 ng $\text{Cl}^{18}\text{O}_4^-$ in 0.50 mL deionized water) to control for ion suppression at the electrospray interface. This solution was subsequently analyzed using ion chromatography-electrospray ionization-tandem mass spectrometry. Perchlorate was quantified based on the peak area ratio of

Table 1. Perchlorate Content of Iceberg and Romaine Lettuce as Affected by Perchlorate Containing Sodium Hypochlorite Treatments

treatment	perchlorate, ^a $\mu\text{g}/\text{kg}$ fw	
	iceberg	romaine
untreated control	7.4 (3.0)	7.1 (4.0)
sodium hypochlorite solution (40 $\mu\text{g}/\text{L}$ ClO_4)	5.5 (1.4)	7.5 (2.0)
sodium hypochlorite solution (40 $\mu\text{g}/\text{L}$ ClO_4)/trim	5.9 (2.0)	9.5 (0.5)
sodium hypochlorite solution (40 $\mu\text{g}/\text{L}$ ClO_4)/wash	8.1 (3.9)	11.9 (3.1)
sodium hypochlorite solution (385 $\mu\text{g}/\text{L}$ ClO_4)	5.8 (0.9)	6.0 (2.1)
sodium hypochlorite solution (385 $\mu\text{g}/\text{L}$ ClO_4)/trim	5.1 (0.3)	3.4 (1.4)
sodium hypochlorite solution (385 $\mu\text{g}/\text{L}$ ClO_4)/wash	5.1 (2.1)	8.2 (5.6)
stat.		
spray	NS	NS
postharvest handling	NS	b

^a NS for $P > 0.05$; values in parenthesis are standard deviations. ^b $P < 0.05$.

analyte to stable isotope-labeled internal standard. A subset of samples were analyzed in duplicate to evaluate assay precision. All percent differences were less than 10%, indicative of acceptable assay precision. Absolute assay accuracy was verified by the blind analysis of four different perchlorate reference solutions (AccuStandard, New Haven, CT); analysis of these proficiency testing solutions across the study time period yielded an average percent difference of -5.2% (CI, -7.2 to -3.2%). The MDL was estimated to be $0.05 \mu\text{g}/\text{L}$ and the MRL was $0.1 \mu\text{g}/\text{L}$. This corresponded to a lettuce perchlorate concentration of approximately $1.3 \mu\text{g}/\text{kg}$ fw.

Water and Hypochlorite Solutions. From all experiments, water samples and hypochlorite solutions were subsampled into 250 mL amber bottles and stored frozen until analysis. The samples were analyzed for perchlorate using the IC/MS/MS methods described above. The values of perchlorate reported for the final hypochlorite solutions are actual measured values.

Statistical Analysis. Analysis of variance was performed by using SAS-GLM and differences were evaluated by F-test (24)

RESULTS AND DISCUSSION

In a small survey of nine hypochlorite samples collected from concentrated solutions used in the produce and dairy industries in Arizona we found perchlorate concentrations ranging from 2580 to 23400 $\mu\text{g}/\text{L}$. This range is generally higher than the range of 89 to 8000 $\mu\text{g}/\text{L}$ previously reported in Massachusetts (19). We found the hypochlorite solutions used in Arizona were generally stored in areas that were not air conditioned. It has been reported that the amount of perchlorate is related to storage conditions, including temperature (20), and this may explain the generally higher concentrations we observed.

Based upon preliminary mass balance calculations, we did not anticipate that using sodium hypochlorite solutions applied as spray or dip treatments to lettuce would be a significant route of perchlorate exposure. The highest concentration of hypochlorite used for treating lettuce is typically 200 mg/L. This involves a 300-fold dilution of a 6% sodium hypochlorite solution. Hence, the final perchlorate concentration of a dilute solution having an initial perchlorate concentration of approximately 24000 $\mu\text{g}/\text{L}$ would be 77 $\mu\text{g}/\text{L}$. Furthermore, less than 5 mL of solution are used for complete coverage of lettuce, thus a lettuce plant would not receive more than $0.38 \mu\text{g}$ of perchlorate. The weight of marketable lettuce ranges from 250 to 900 g depending on lettuce type and trim (8). However, even for the smaller plant weights, the final concentration of the perchlorate added to lettuce from hypochlorite would approach $1.2 \mu\text{g}/\text{kg}$ fw, which is our reporting level by IC/MS/MS.

This inference was confirmed by field studies in which perchlorate contents of lettuce were not significantly ($P > 0.1$) increased by hypochlorite treatments (Table 1). There seemed to be a significant ($P < 0.03$) difference to postharvest washing

Table 2. Perchlorate Content of Romaine Lettuce as Affected by Perchlorate Containing Sodium Hypochlorite Treatments

treatment	perchlorate, ^a $\mu\text{g}/\text{kg}$ fw
control	58.5 (4.3)
control/trim	48.7 (23.9)
sodium hypochlorite solution (54 $\mu\text{g}/\text{L}$ ClO_4)	70.5 (19.3)
sodium hypochlorite solution (54 $\mu\text{g}/\text{L}$ ClO_4)/trim	37.3 (12.1)
sodium hypochlorite solution (237 $\mu\text{g}/\text{L}$ ClO_4)	66.3 (20.2)
sodium hypochlorite solution (237 $\mu\text{g}/\text{L}$ ClO_4)/trim	57.4 (19.1)
stat.	
spray	NS
trim	b

^a NS for $P > 0.05$; values in parenthesis are standard deviations. ^b $P < 0.05$.

Table 3. Perchlorate Levels in Lettuce Following Hypochlorite Treatments during Salad Processing

treatment	perchlorate concentration ^a ($\mu\text{g}/\text{kg}$ fw)
control	15.5 (8.2)
200 mg/L spray	12.9 (3.2)
200 mg/L spray/60 mg/L dip	13.8 (3.9)
200 mg/L spray/60 mg/L dip/20 mg/L dip	14.2 (5.7)
stat.	NS

^a NS for $P > 0.05$; values in parenthesis are standard deviations.

of the romaine lettuce which is difficult to explain because perchlorate in the well water used for washing was below detection by LC/MS/MS. Data from the first field study showed more variation for romaine than iceberg lettuce. We had suspected the variation was associated with subsampling error after shredding, however, we did not reduce this variation with more rigorous mixing in the second field experiment. As we have shown in previous studies (8), there is a decreasing gradient in perchlorate concentration from outer to inner leaves. We suspect small sampling errors were responsible for this variation. Nonetheless, even the highest perchlorate spiked hypochlorite treatment did not significantly increase the perchlorate contents of iceberg ($P > 0.19$) and romaine ($P > 0.12$) lettuce relative to the untreated controls.

The background perchlorate values in the second field experiment are higher than those in the first field study because this experiment was conducted in the lower Colorado River region where trace levels of perchlorate accumulate with the uptake and transpiration of Colorado River water (8). Similar to the first field experiment, there were no significant ($P > 0.34$) differences in lettuce perchlorate concentrations to hypochlorite treatment (Table 2). However, differences to trimming were significant ($P < 0.01$), reflecting a reduction associated with removing outer leaves that have higher perchlorate concentrations.

The background perchlorate values for iceberg lettuce in the simulated salad treatment study were also marginally higher than those in the first field study because this experiment was also conducted with lettuce produced in the lower Colorado River (Table 3). There were no significant ($P > 0.76$) increases in perchlorate attributable to treatment with perchlorate containing hypochlorite solutions (Table 3).

Overall, these results show that using hypochlorite solutions that contain perchlorate, even 10-fold more than the highest levels observed in surveys, did not significantly increase the perchlorate content of iceberg or romaine lettuce. Because of the relatively low concentrations of perchlorate present after dilution and the low volumes applied to lettuce in sprays or dips, hypochlorite solutions are not a significant source of the perchlorate levels found in lettuce.

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NOTE ADDED AFTER ASAP PUBLICATION

After this paper was published ASAP February 20, 2009, additional changes were made; the corrected version was reposted February 26, 2009.

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