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Perchlorate, Thiocyanate, and Nitrate in Edible Cole Crops (*Brassica* sp.) Produced in the Lower Colorado River Region

C. A. Sanchez · B. C. Blount · L. Valentin-Blasini · R. I. Krieger

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Abstract The Colorado River is contaminated with low levels of perchlorate. Perchlorate has the potential to disrupt thyroid function by inhibiting the uptake of iodide. *Brassica* are rich sources of thiocyanate and nitrate, also inhibitors of iodide uptake. This study was conducted to estimate potential human exposure to perchlorate, thiocyanate, and nitrate from *Brassica* sp. irrigated with Colorado River water. Results indicate that *Brassica* sp. irrigated with Colorado River water do accumulate trace levels of perchlorate. However, the levels of perchlorate observed are low relative to the nitrate and thiocyanate naturally present in these species and low relative to the reference dose recommended by the NAS and the USEPA.

Keywords Perchlorate exposure · Thiocyanate · Nitrate · Thyroid · Goitrogens

Perchlorate has the potential to cause thyroid dysfunction by inhibiting iodide uptake by the sodium iodide symporter (NIS) (Clark 2000). There is concern that perchlorate-contaminated waters may represent a health risk as sources of drinking water to humans and irrigation water to food crops. However, a number of other inorganic anions present in some food crops, including nitrate and thiocyanate, can act as goitrogens by blocking iodide uptake of the NIS in a competitive manner (Wyngaarden et al. 1953; Tonacchera et al. 2005).

Perchlorate in the lower Colorado River has ranged from 2 to 9 μg/L and was introduced into Lake Mead through contamination from a perchlorate salt manufacturing plant near the Las Vegas Wash (DHS 2000). The production of fresh market vegetables in the lower Colorado regions of Arizona and California is a 2 billion dollar industry. This industry relies on Colorado River water for irrigation and there is concern that consumption of perchlorate through food produced in the region may represent a significant source of exposure. Several plant species have been shown to absorb perchlorate from soil and irrigation water (Tan et al. 2004; Yu et al. 2004) and there is evidence that perchlorate accumulates in certain food crops in general (Jackson et al. 2005; Sanchez et al. 2005a), and in the lower Colorado River region in particular (Sanchez et al. 2005b, 2006). However, thiocyanates and nitrate are also present in *Brassica* sp. (Kushad et al. 1999). The objectives of this project was to estimate potential human exposure to perchlorate from broccoli (*Brassica oleracea* L., var *italica*), cauliflower (*B. oleracea* L., var. *botrytis*), and cabbage (*B. oleracea* L., var. *capitata*) produced in the lower Colorado River region, and estimate the possible biologic significance of perchlorate relative to thiocyanate and nitrate in these edible crops.

C. A. Sanchez (✉)
Department of Soil, Water, and Environmental Sciences, Yuma Agricultural Center, The University of Arizona, Yuma, AZ 85364, USA
e-mail: Sanchez@ag.arizona.edu

B. C. Blount · L. Valentin-Blasini
National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), Atlanta, GA 30341, USA

R. I. Krieger
Personal Chemical Exposure Program, Department of Entomology, University of California, Riverside, CA 92521, USA

Materials and Methods

All fields selected for sampling were irrigated with water from the Colorado River. Areas sampled included the

Coachella Valley and Imperial Valley of California and the Lower Colorado River Valley of California and Arizona. Samples were collected during production seasons from 2003 to 2005. Edible portions were diced, mixed thoroughly, and a sub-sample was placed in a freezer. The frozen samples were freeze–dried as space became available on a freeze drier and weights recorded before and after freeze-drying. The samples were ground and stored in vials for extraction.

We used an extraction procedure where 600 mg of freeze–dried product was weighed into centrifuge tubes, 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 (Ellington and Evans 2000). The tubes were then centrifuged for 30 min and the supernatants filtered through 0.2 μm Gel-man ion membrane syringe filters. Two milliliters of the above extract (extract one) was reacted with 1,000 mg DD-alumina. Vials were gently agitated two or three times over a 24-h period. Eighteen milliliters of DI water were then added to this 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 for perchlorate and thiocyanate by ion chromatography/tandem mass spectroscopy (IC/MS/MS) using stable isotope labeled internal standard methodology reported previously (Valentin-Blasini et al. 2005). Briefly, 0.5 mL of aqueous sample extract was spiked with an isotopically labeled internal standards ($Cl^{18}O_4^-$, $SC^{15}N^-$) and diluted 1:1 with deionized water. This solution was subsequently analyzed using ion chromatography–electrospray ionization–tandem mass spectrometry. Perchlorate and thiocyanate were quantified based on the peak area ratio of analyte to stable isotope-labeled internal standard. A subset of samples (10%) were analyzed further using standard addition, and produced acceptable percent differences of <10%. Absolute assay accuracy was verified by the blind analysis of four different reference solutions containing perchlorate and thiocyanate (AccuStandard, New Haven, CT, USA); analysis of these proficiency testing solutions across the study time period yielded an average percent difference of ±5.2%. The perchlorate MDL was estimated to be 0.02 μg/L and the MRL was 0.1 μg/L. The percentage dry matter of the edible portions of broccoli, cauliflower, and cabbage were 13%, 9%, and 9%, respectively. Therefore, an MRL of 0.1 μg/L by IC/MS/MS would correspond to approximately 2–3 μg/kg fw. We used median perchlorate and thiocyanate concentrations in the edible crops and mean and 90th percentile consumption estimates to estimate exposures. The data for broccoli and cabbage were taken from Smiciklas-Wright et al. (2002) and the data for cauliflower were unpublished data provided to the authors by Exponent.

Nitrate in freeze–dried plant tissue was determined potentiometrically (Baker and Smith 1969). Approximately 400 mg of tissue and 0.04 L of $Al_2(SO_4)_3$ buffer solution were placed in 250 mL Erlenmeyer flasks, put on a shaker for 30 min and filtered. The filtrates were analyzed for nitrate using a calibrated nitrate selective electrode and potentiometer.

Results and Discussion

Perchlorate concentrations in the irrigation water ranged from 2 to 9 μg/L during the sampling period (Sanchez et al. 2005b, 2006). Levels of perchlorate in edible plant material ranged from 3.5 to 106.9 μg/kg fw, 4.2–40.7 μg/kg fw, and 4.6–63.2 μg/kg fw in broccoli, cauliflower, and cabbage, respectively (Table 1). Perchlorate, thiocyanate and nitrate concentrations were significantly higher in broccoli compared to cauliflower and cabbage (Table 1). Thiocyanate concentrations were two to three orders of magnitude higher and nitrate concentrations were approximately five to six orders of magnitude higher than perchlorate.

Mean hypothetical perchlorate exposure for average individuals across age and gender was 1.6, 0.05, and 0.95 μg/person for broccoli, cauliflower, and cabbage, respectively (Table 2). Estimated perchlorate exposures for cauliflower and cabbage are less than broccoli because concentrations are lower than broccoli, and the consumption of cauliflower and cabbage is less than broccoli.

Perchlorate in edible broccoli was used to estimate potential exposure and dosage across gender and age (Table 3). Even for broccoli, regardless of age and gender, the estimated dosages are less than 0.1% the reference dose of 0.7 μg/kg per day adopted by the USEPA (2005) on recommendation from the National Academy of Science (2005). This reference dose is based on a non-observed effect level (NOEL) of 7 μg/kg per day from a human perchlorate dosing study to which a tenfold uncertainty factor was applied to address potential sensitive subpopulations (Greer et al. 2002). Interestingly, these estimated perchlorate dosages for adults from broccoli were less than 13% of the 95th percentile of estimated total dosage of 0.234 μg/kg per day for the adult US population (Blount et al. 2006).

Another consideration with respect to the biological significance of perchlorate exposure is the presence of other natural goitrogens in food (Belzer et al. 2004). The iodine uptake inhibiting potential of a mixture of inhibitors can be forecast using perchlorate equivalent concentration (PEC). The PEC is defined as the sum of the concentrations of the inhibitors present divided by their inhibition potency

Table 1 Range, mean, median, and 90th percentile levels of perchlorate, thiocyanate, and nitrate found in the edible portions of broccoli, cauliflower, and cabbage

Crop	n	Min.	Max.	Mean	Median	90th percentile
Perchlorate (μg/kg fw)						
Broccoli	55	3.5	106.9	23.8	19.3	37.2
Cauliflower	38	4.2	40.7	12.8	11.2	23.7
Cabbage	19	4.6	63.2	18.5	15.3	41.1
Thiocyanate (μg/kg fw)						
Broccoli	55	1965	56,219	17,917	15,030	36,664
Cauliflower	38	1391	28,587	9,206	7,871	19,085
Cabbage	19	1712	20,129	7,659	6,974	15,153
Nitrate[a] (μg/kg fw)						
Broccoli	55	579,000	6,087,000	1,717,000	1,503,000	3,174,000
Cauliflower	38	353,000	1,938,000	1,037,000	998,000	1,647,000
Cabbage	19	429,000	1,786,000	1,001,000	1,072,000	1,458,000

[a] Rounded to 1,000 μg/kg fw

Table 2 Hypothetical exposures for members of the US population whom consume broccoli, cauliflower, and cabbage

Crop	Consumption[a,b] (g/day)	Perchlorate intake[b] (μg/day)
Broccoli	81 (183)	1.56 (3.53)
Cauliflower	4.7 (13.1)	0.05 (0.15)
Cabbage	62 (149)	0.95 (2.28)

[a] All individuals age 2 and older

[b] Values in parenthesis are 90th percentile values. Intake (μg perchlorate/day) = concentration (μg/kg fresh weight) × consumption (kg/day)

relative to perchlorate on a weight ingestion basis. Recent work has shown that the relative potency of perchlorate to inhibit iodide uptake at the NIS was 15 times that of thiocyanate and 240 times that of nitrate (Tonacchera et al. 2005). However, because thiocyanate has a serum half-life 18–29 times that of perchlorate and nitrate, the corresponding relative potencies on an ingested-weight basis were calculated 0.5 and 240 for thiocyanate and nitrate, respectively. Thus, the PEC of a food containing perchlorate, thiocyanate, and nitrate can be estimated as the concentration of perchlorate plus 1/0.5× thiocyanate and 1/240× of the concentration of nitrate.

Table 4 shows the PEC calculated using the average concentrations of perchlorate, thiocyanate, and nitrate in the *Brassica* sp. samples collected. The iodine uptake inhibition potential from perchlorate is less than 0.1% that estimated from all measured iodide uptake inhibitors combined. There is uncertainty about how thiocyanates are extracted from plant residues using our methodology relate

Table 3 Hypothetical mean and 90th percentile exposure of children and adults whom consume broccoli produced in the lower Colorado River region

Gender	Age (years)	Body weight (kg)	Consumption[a] (g/day)	Intake[a,b] (μg/day)	Dosage[a] (μg/kg body weight day)
Males and females	2–5	16	49 (92)	0.95 (1.78)	0.06 (0.11)
Males and females	6–11	29	67 (177)	1.29 (3.42)	0.04 (0.12)
Males	12–19	58	82 (182)	1.58 (3.51)	0.03 (0.06)
Females	12–19	53	82 (183)	1.58 (3.53)	0.03 (0.06)
Males	20–39	77	114 (233)	2.20 (4.50)	0.03 (0.06)
Females	20–39	61	77 (159)	1.49 (3.07)	0.02 (0.05)
Males	40–59	78	90 (183)	1.74 (3.53)	0.02 (0.05)
Females	40–59	65	65 (154)	1.25 (2.97)	0.02 (0.05)
Males	>60	74	82 (183)	1.58 (3.53)	0.02 (0.05)
Females	>60	65	78 (155)	1.51 (2.99)	0.02 (0.05)

[a] Values in parenthesis are 90th percentile values

[b] Exposure estimates were made using consumption data sets (Smiciklas-Wright et al. 2002) and body weights factors (USEPA 1998) compiled by others. Dosage (μg/kg body weight) = exposure (μg/day)/body weight (kg)

Table 4 The perchlorate equivalent concentrations (PECs) of anions in broccoli, cauliflower, and cabbage produced in the lower Colorado River region

Crop	Perchlorate (μg/kg fw)	PEC of thiocyanate (μg/kg fw)	PEC nitrate (μg/kg fw)	Total PEC[a] (μg/kg)
Broccoli	19.3	30,060	6,263	36,342
Cauliflower	11.2	15,742	4,158	19,912
Cabbage	15.3	13,948	4,467	18,429

[a] Total PEC = (ClO_4) + (thiocyanate)/0.5) + (nitrate/240)

to that released upon ingestion. Studies with animals and humans have shown serum and urine thiocyanate levels increase with consumption of *Brassica* sp. (Cox-Ganser et al. 1994; Olea and Parras 1992; Tripathi et al. 2001). Therefore, even if our extraction procedure over-estimated the thiocyanate released upon ingestion by two orders of magnitude, the thiocyanate and nitrate would be more likely to inhibit NIS than the perchlorate found in these *Brassica* species. This observation is consistent with previous observations we have made with nitrate and perchlorate in lettuce (*Lactuca sativa* L.) and underscore the paradox of assigning risk to the trace levels of perchlorate associated with consumption of food crops produced in the lower Colorado River region (Sanchez et al. 2005b).

In conclusion, *Brassica* sp. irrigated with Colorado River water do accumulate trace levels of perchlorate. However, the levels of perchlorate observed are low relative to the nitrate and thiocyanate naturally present in these food plants and low relative to the reference dose recommended by the NAS and the USEPA.

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Disclaimer The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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