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Perchlorate and Nitrate in Leafy Vegetables of North America

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In previous studies trace levels of perchlorate were found in lettuce (*Lactuca sativa* L.) irrigated with Colorado River water, which is contaminated with low levels of perchlorate from aerospace and defense related industries. In this paper, we report the results of a survey conducted across North America to evaluate the occurrence of perchlorate in leafy vegetables produced outside the lower Colorado River region, and evaluate the relative iodide uptake inhibition potential to perchlorate and nitrate in these leafy vegetables. Conventionally and organically produced lettuce and other leafy vegetable samples were collected from production fields and farmers' markets in the central and coastal valleys of California, New Mexico, Colorado, Michigan, Ohio, New York, Quebec, and New Jersey. Results show that 16% of the conventionally produced samples and 32% of the organically produced samples had quantifiable levels of perchlorate using ion chromatography. Estimated perchlorate exposure from organically produced leafy vegetables was approximately 2 times that of conventional produce, but generally less than 10% of the reference dose recommended by the National Academy of Sciences. Furthermore, the iodide uptake inhibition potential of perchlorate was less than 1% of that of the nitrate present. These data are consistent with those of other reported perchlorate survey work with lettuce, bottled water, breast milk, dairy milk, and human urine, and suggest a wide national presence of perchlorate.

Introduction

Perchlorate has been discovered in surface water and groundwater supplies throughout the United States. There is concern that these perchlorate-contaminated waters may represent a health risk both as sources of drinking water and irrigation water for food crops. Perchlorate has the potential to cause thyroid dysfunction by inhibiting iodide uptake by

the sodium iodide symporter (NIS) (1). Perchlorate contamination in the Colorado River is well documented and is introduced into Lake Mead by a perchlorate salt manufacturing plant previously located near the Las Vegas wash. It has been reported that the Colorado River below Lake Mead has had concentrations ranging from 5 to 9 $\mu\text{g/L}$ (2). In contrast, perchlorate found in groundwater sources in the Southern High Plains of Texas in concentrations ranging from 4 to 60 $\mu\text{g/L}$ (3) have been, at least partially, attributed to natural atmospheric processes (4).

Fertilizer has been considered a potential source of perchlorate for plants. A recent comprehensive evaluation of inorganic fertilizer has shown that, with the exception of products derived from Chilean nitrate, fertilizers are not a significant source of perchlorate to the environment (5). A limited amount of Chilean nitrate has been used historically in vegetable, fruit, and tobacco production systems. More recently, it has frequently been used in organic production systems, as it is the only form of mineral N allowed (6). However, it is estimated that less than 0.1% of the N fertilizer used in the United States is derived from Chilean nitrate. A recent survey by the USGS has found perchlorate in other amendments (7), including blood meal, fish meal, and kelp.

Several plant species have been shown to absorb and accumulate perchlorate from soil and irrigation water. Perchlorate has been detected in several plant species (8, 9) growing in the Las Vegas wash. Accumulation of perchlorate in tobacco fertilized with perchlorate containing Chilean nitrate has also been reported (10). There is evidence that perchlorate accumulates in certain food crops. This was initially inferred from studies with pertechnetate, which is chemically similar to perchlorate and is often considered an analogue for the study of perchlorate (11). Numerous studies have shown that both perchlorate and its analogue pertechnetate are absorbed and accumulate in leaves, but not to the same extent in the fruit (12–15).

Accumulation of perchlorate in crops where leaves are consumed potentially represents an important source of exposure. At the request of the Environmental Working Group (EWG), The Institute of Environmental and Human Health of Texas Tech University analyzed 22 leafy vegetable samples purchased in California in the winter of 2002–2003 (16). Presumably these samples were grown in the lower Colorado River region since they were purchased in the winter months when most of the leafy produce is shipped from this region. Four of the 22 samples contained detectable levels of perchlorate, and one of these (organic baby greens) was quantifiable at 121 $\mu\text{g/kg}$ on a fresh weight (fw) basis. More recently, a preliminary FDA "market basket" survey found perchlorate in lettuce irrigated with Colorado River water to range from below quantifiable levels to 129 $\mu\text{g/kg}$ fw (17). Interestingly, the few organically produced samples collected by the EWG and the FDA had among the highest perchlorate concentrations observed (4). A more extensive survey of lettuce in the lower Colorado River region reported perchlorate concentrations ranging from below detection to 104 $\mu\text{g/kg}$ fw, and exposure estimates ranging from 0.45 to 1.8 $\mu\text{g/day}$ depending on lettuce type and trimming (18). For all lettuce types in this survey, hypothetical exposures were less than 4% of the reference dose recommended by the National Academy of Sciences (19).

Nitrate, and other inorganic ions, can block iodide uptake of the sodium iodide symporter (NIS) in a competitive manner (20, 21). Considering molecular weights and serum half-lives,

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TABLE 1. Sample Collection Dates and Regions during 2004

sample collection date	region sampled
April 23–24	Central Valley, California
May 6–7	Rio Grande Valley, southern New Mexico
June 25–30	Michigan
June 30–July 3	Ohio
July 5–6, 8–11, 17–18	New York
July 6–7	Quebec
July 17, October 1–10	New Jersey ^a
July 21–24	San Luis Valley, southern Colorado & northern New Mexico
June 25–Oct 15	coastal valleys, California ^b

^a All of the samples collected from New Jersey in the fall were collected by Dr. Wesley Kline of Rutgers University. ^b Because most lettuce consumed in the United States during the late spring, summer, and early fall months is produced in the coastal valleys of California we made multiple collections from these areas. The samples that the corresponding author could not collect personally in this area of California were collected by Dr. H. Ajwa and Dr. M. Hogue of UC-Davis.

perchlorate has been calculated to be 240 times more potent at blocking iodide uptake than nitrate on an ingested weight basis (21). Although nitrate is less potent as an iodide uptake inhibitor than perchlorate, it is naturally present in much higher concentrations in most leafy vegetables (18, 22, 23). In one survey (18), the relative potential iodide uptake inhibition potential due to lettuce nitrate was 2 orders of magnitude greater than that associated with the corresponding trace levels of perchlorate.

The FDA reported perchlorate in lettuce and dairy milk samples collected from production regions outside the lower Colorado River region ranging from below quantification to 72 $\mu\text{g/kg}$ fw and 11.3 $\mu\text{g/L}$, respectively. Recent work has reported perchlorate in dairy milk and women's breast milk (24) nationally and in human urine (25) in Atlanta, suggesting perchlorate may be more ubiquitous than previously thought. A recent study detected perchlorate in a majority of the bottled water samples evaluated and concluded that perchlorate is a ubiquitous contaminant of natural waters at trace levels (26). The objective of the present study was to survey lettuce and other leafy vegetables produced in various regions across North America for perchlorate and nitrate content, and compare concentrations for leafy vegetables grown under conventional and organic production systems.

Materials and Methods

Sample Collection. In most instances the corresponding author personally collected samples in grower's fields and/or from farmers markets, but in a few instances sample collections were made by professional colleagues at the University of California-Davis and Rutgers University. He traveled to production regions and/or farmers' markets, or arranged for the collection of product, in all areas during the harvest season. Because we have previously reported concentrations and exposure from lettuce produced in the lower Colorado River region (18), this survey was restricted to areas outside the lower Colorado River region. Sample collection dates and regions are listed in Table 1.

Sampling was focused on various types of lettuce (*Lactuca sativa* L.), but in some production regions other types of leafy vegetables were equally or more prevalent. Therefore, in many instances we substituted or augmented lettuce samples with other types of leafy vegetables (Table 2). We trimmed all iceberg lettuce for wrapping, which is the more common trim sold commercially. This involved the removal of a few extra wrapper leaves compared to a naked trim (18). All other lettuce types were harvested for a naked trim, as they are commonly marketed.

Plant samples were diced and mixed thoroughly, and a sub-sample was placed in a freezer. The samples were shipped overnight on dry ice from the sampling location to the Soil, Water, and Environmental Science Research Laboratory at the University of Arizona's Yuma Agricultural Center.

Sample Processing and Extraction for Perchlorate. The frozen samples were freeze-dried on a Labconco freeze-drier, typically requiring 48 h for complete freeze-drying. Weights before and after freeze-drying were recorded, and the samples were subsequently ground and stored in vials for extraction. We used an extraction procedure described previously (27) with minor modifications. Briefly, 600 mg of freeze-dried product was weighed into centrifuge tubes and 15 mL of DI water was 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 were filtered sequentially through Kim wipes and 0.2- μm Gel-man ion membrane syringe filters. A 2-mL portion of the above extract (extract 1) was reacted with 1000 mg of DD-alumina. Vials were gently agitated 2 or 3 times over a 24-hour period after which 18 mL of DI water was added to the mixture. After the solution was stirred and settled, it 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 (IC). Before loading on the IC, the extracts were allowed to reach room temperature and were filtered through preconditioned Dionex "On Guard" RP syringe filters. Furthermore, the first 0.75 mL of sample (extract 2) pushed through the filter was discarded and the remaining aliquots were used for IC analysis.

Perchlorate Analysis. All perchlorate analyses were performed on a Dionex 2500 consisting of an IP 25 isocratic pump, an EG50 eluent generator, a continuous regenerating trap column, a CD 25 conductivity detector, the 2-mm AG16/AS16 guard and separation column pair, and an AMMS III suppressor. The columns, suppressor, and detector are housed in a LC 30 chromatography oven. We used 50 mM KOH eluent and 50 mM sulfuric acid suppression. A 1000- μL injection loop was used and elution time ranged from 9.5 to 11.0 min. Calibration was performed with standards ranging from 0.5 to 100 $\mu\text{g/L}$. Ideally, calibration should be done in matrix, however, because matrixes are not constant in environmental and biological specimens, this is difficult to do. Therefore, we guarded against matrix errors by spiked additions. A minimum of 10% of the samples were extracted with a 100 $\mu\text{g/L}$ perchlorate standard to yield 10 $\mu\text{g/L}$ perchlorate after dilution. The method detection limit (MDL) was determined using the procedure outlined in EPA method 314.0 (28) using seven replicates of a standard in reagent water. The calculated MDL was 0.2 $\mu\text{g/L}$ using a 0.5 $\mu\text{g/L}$ standard. We set the minimum reporting level (MRL) for leafy vegetable extracts at 1.5 $\mu\text{g/L}$. As a standard practice we would run 10% duplicate extractions in addition to the 10% spiked additions. Duplicate aliquots of a given extraction were always analyzed. Additional aliquots or replicates were analyzed if we judged variability on the first two aliquots or replicates excessive. In addition, duplicate extracts for approximately 10% of all samples were sent out to a commercial laboratory for confirmatory analysis by IC/MS/MS using an ^{18}O internal standard methodology similar that that reported by others (25, 26). The samples sent out for confirmatory analysis included those with problematic matrixes and samples with abnormally high values, as well as samples selected at random. A comparison of perchlorate concentrations and estimates determined by IC with duplicate extracts sent out for IC/MS/MS confirmatory analysis show excellent agreement (slope 1.02 and R^2 of 0.98).

TABLE 2. Types and Number of Leafy Vegetable or Sprouts Sampled, and Mean and Range of Perchlorate Concentrations Observed Nationally

leafy crop	botanical name	samples collected	mean $\mu\text{g/kg fw}$	min. $\mu\text{g/kg fw}$	max. $\mu\text{g/kg fw}$
iceberg lettuce	<i>Lactuca sativa</i> L.	63	7.4	<DL	31
romaine lettuce	<i>Lactuca sativa</i> L.	84	17.1	<DL	100
green leaf lettuce	<i>Lactuca sativa</i> L.	69	16.5	<DL	195
red leaf lettuce	<i>Lactuca sativa</i> L.	67	14.5	<DL	104
butter head lettuce	<i>Lactuca sativa</i> L.	45	17.2	<DL	98
baby mix ^a	<i>Lactuca sativa</i> L.	44	27.0	<DL	370
endive	<i>Chichorium endiva</i> Lam	9	22.8	<DL	49
escarole	<i>Chichorium endiva</i> Lam	13	13.1	<DL	33
arugula	<i>Eruca sativa</i> Miller	9	55.8	<DL	195
spinach	<i>Spinacia oleracea</i> L.	10	85.1	<DL	628
basil	<i>Ocimum basilicum</i> L.	1	242	242	242
dandelions	<i>Taraxacum officinale</i> Wiggers	4	<DL	<DL	<DL
sunflower sprouts	<i>Helianthus annuus</i>	2	<DL	<DL	<DL
kale	<i>Brassica oleracea</i> L. (var. sabellica) L.	7	20.3	<DL	112
mustard greens	<i>Brassica juncea</i> (var. sspp.)	4	<DL	<DL	<DL
beet tops	<i>Beta vulgaris</i>	2	<DL	<DL	<DL
mibuna	<i>Brassica rapa</i> (var. nipposinica)	1	<DL	<DL	<DL
tatsoi	<i>Brassica rapa</i> (var. rosularis)	1	5	<DL	5
chards	<i>Beta vulgaris</i> (var. flavesces) Lam	1	5	<DL	5
collards	<i>Brassica oleracea</i> L. (var. viridis) L.	1	5	<DL	5
parsely	<i>Petroselinum crispum</i> Mill.	1	74	74	74

^a Certain blends of baby mix sometimes contain some spinach, dandelions, flowers, or some leafy *Brassica*.

Nitrate Analysis of Plant Tissue. Nitrate in freeze-dried plant tissue was determined potentiometrically (29). Approximately 400 mg of tissue and 0.04 L of $\text{Al}_2(\text{SO}_4)_3$ 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. There was insufficient sample after perchlorate analysis for nitrate analysis for six samples.

Exposure Estimates. As noted previously, our reporting level for perchlorate was set at 1.5 $\mu\text{g/L}$ for leafy vegetable extracts. Depending on the moisture content of the leafy vegetable and based on extraction and dilution ratios employed, this corresponded to approximately 20–40 $\mu\text{g/kg fw}$ depending on leafy vegetable type. Thus, for exposure estimates we estimated 17.5 $\mu\text{g/kg}$ for all leafy vegetable types below the MRL. We used an estimate of 10 $\mu\text{g/kg}$ for all levels below detection. For calculations of exposure and dosage we assumed a daily consumption of lettuce or leafy vegetable of 55 g (30) and an average adult body weight of 70 kg.

Statistical Analysis. Statistical tests for leafy type and the difference between organic and conventional culture in the percent of samples above the MRL was conducted using Fisher's Exact Test (31). A regression analysis was conducted in which the logarithms of perchlorate sample concentrations were assumed to be normally distributed with common variance, and means that depended possibly on lettuce type, culture (organic versus conventional), and state or province, but with no interaction between culture and state or province. In this analysis leafy types with fewer than 9 observations were combined. The log-transformed data were consistent with a normal distribution ($p = 0.40$) according to the Shapiro–Wilk test (32). Because of the large percentage of samples with perchlorate below the detection limit (DL), or between the DL and the MRL, this analysis was implemented using a likelihood approach in which a nondetect sample provided a term in the likelihood equal to the probability a sample value was below the DL of 10 $\mu\text{g/kg}$, and a sample value below the MRL but above the DL provided a term in the likelihood equal to the probability that the sample value was between 10 and 25 $\mu\text{g/kg fw}$ (33). This method does not require assigning values to samples with values below the MRL. For comparison, a conventional regression analysis was also applied in which sample values below the detection

limit were assigned a value of one-half the detection limit and values below the MRL and above the detection limit were assigned a value of 17.5 $\mu\text{g/kg}$ (34).

Results and Discussion

The total number of samples collected, and the mean and range of perchlorate concentrations for all leafy vegetable types is shown in Table 2. Iceberg lettuce had significantly lower perchlorate concentrations compared to other leafy vegetable types evaluated when variation due to state was not partitioned. As noted in a previous study (18) transpiration in iceberg lettuce largely occurs in the outer leaves, many of which are trimmed by the harvester, grocer, and consumer. Conversely, other lettuce types (and other leafy vegetables) have a more open leaf structure compared to iceberg lettuce, resulting in transpiration in more of the leaves, thereby resulting in more perchlorate accumulation in more of the edible portion. Both state and culture were highly significant in the regression analysis, with state more significant than culture. When state and culture were controlled for there were no statistically significant differences due to leafy type ($P = 0.102$ 10 df), which suggests that variation across sampling locations was greater than differences among lettuce types.

The total number above the MRL, the mean and range of those above the MRL, the number between the MRL and detections, and the number below detection by state or province and culture is shown in Table 3. The percentage of samples from conventional culture with values below the MRL (84%) was significantly higher than that from organic culture (68%) ($p < 0.00001$, Fishers Exact Test). The average perchlorate concentration from organic culture was 34.7 $\mu\text{g/kg fw}$, compared to 16.4 $\mu\text{g/kg fw}$ from conventional culture. Similarly, in the regression analysis, the median sample concentration from organic culture was estimated to be 2.2 times higher than that from conventional culture ($p < 0.00001$).

Although the data also indicate statistical differences among states, we do not consider comparisons among states meaningful due to the different manner by which samples were collected in different states, the large difference in the number of samples collected in each state, and observed

TABLE 3. Summary of Perchlorate Contents in Leafy Vegetables by State and Culture

state	total number	samples > MRL	Quantifiable (µg/kg fw)		number <MRL and >DL	number <DL
			mean	range		
Conventional						
California	88	10	41.3	22–75	10	68
Colorado	6					6
New Jersey	38	22	47.9	23–104	10	6
New Mexico	9					9
New York	30	2	33.5	27–40	0	28
Michigan	35	3	28.0	18–46	2	30
Ohio	43	4	66.5	44–84	4	35
Quebec	19	1	36.0	36–36	2	16
total	268	42			28	198
Organic						
California	54	18	72.5	21–370	7	29
Colorado	13					13
New Jersey	11				4	7
New Mexico	18	6	183.2	24–628	1	11
New York	33	11	61.0	21–110	3	19
Michigan	20	3	130.0	36–242		17
Ohio	16	12	39.1	28–65	4	
Quebec	5	2	44.5	21–68	1	2
total	170	52			20	98
grand total	438	94			48	294

differences within states. For example, in Colorado where we observed no detectable perchlorate, and therefore a statistically significant difference from other states, all samples were collected from production fields in the San Luis Valley, near Alamosa. This is the only major commercial lettuce production area in Colorado and no samples were collected from farmers' markets. However, in Quebec, samples were collected exclusively from farmers' markets because we did not have the professional contacts to gain access to production fields. In most other regions both production fields and farmers' markets were sampled but there were apparent differences within the state. For example, in California, we observed no detectable perchlorate in the spring production region of the Central Valley (Huron) but obtained detectable perchlorate in samples collected in some production fields in the coastal valleys and in product obtained from farmers markets. We observed no detectable perchlorate in production fields in the Mesilla Valley of southern New Mexico but obtained several samples with detectable perchlorate from various small growers obtained at a farmers market in northern New Mexico. Perhaps more important than differences between states is the observation that detectable perchlorate was found in leafy vegetables across several production areas in North America outside of the lower Colorado River region. Similar observations were made in a recent national survey (17). While most of the lettuce consumed in North America during the late fall, winter, and early spring period is produced in the lower Colorado River region of southern California and southwestern Arizona, most of the lettuce consumed in North America during the late spring, summer, and early fall periods is produced in central and northern California. The other regions sampled in this survey included smaller late spring, summer, and early fall leafy production areas. Overall, the amounts of perchlorate found in California outside the lower Colorado River region are similar to amounts found in the other smaller production regions of North America (Figure 1).

Estimated mean perchlorate exposure for adults was approximately 1 $\mu\text{g/day}$ and 2 $\mu\text{g/day}$ for conventional and organically produced leafy vegetables, respectively (Table 4). This is very similar to the 0.45–1.8 $\mu\text{g/day}$ perchlorate exposure estimated from a survey in the lower Colorado River region (18). In the lower Colorado River region the proportion

of perchlorate detections was greater, which is not surprising considering that all lettuce samples collected were irrigated with water contaminated with perchlorate. However, for the national survey we occasionally observed concentrations much higher than those observed in the lower Colorado River region, especially among organically grown produce. The survey in the lower Colorado River region did not include organically produced lettuce.

We do not know the reason for the statistically higher perchlorate contents in organically produced leafy vegetables. Chilean nitrate is currently allowed for use for up to 20% the total N requirement of organically grown crops by the USDA certification program (6) and the perchlorate content in Chilean nitrate ranges from 0.05 to 0.2% on a weight basis (35). However, many organic growers decline to use it because it is not allowed in many international certification programs (36). Consequently, we do not believe the use of Chilean nitrate fully accounts for this observed difference in the perchlorate content of organically and conventionally produced leafy vegetables. Perhaps other products such as kelp and fish-based products (7) are contributing factors as well. Kelp and fish-based products are used by both organic and conventional producers, but we suspect they are more often used by organic producers because conventional producers have alternative products to use for nutrition and complexing agents. In addition, the possibility of natural sources of perchlorate entering the food chain, regardless of culture, cannot be discounted (4). Additional work is needed to identify probable anthropogenic and natural sources of perchlorate entering the food chain.

Although organically grown leafy vegetables potentially provide more than double the perchlorate exposure of conventionally produced leafy vegetables, the estimated exposures in all age and gender groups are at most 10% of the reference dose of 0.7 $\mu\text{g/kg day}$ recommended by the National Academy of Sciences (NAS) (Table 4). The NAS reference dosage is based upon a no-observed effect level of 7 $\mu\text{g/kg}$ from human iodide uptake studies (37) to which a 10-fold uncertainty factor was applied to address all potentially sensitive subpopulations (19).

Another consideration in assessing the risk of perchlorate is its presence relative to nitrate (18, 38). A number of studies have determined the relative potency of perchlorate and nitrate as iodide uptake inhibitors of the NIS (20, 39, 40).

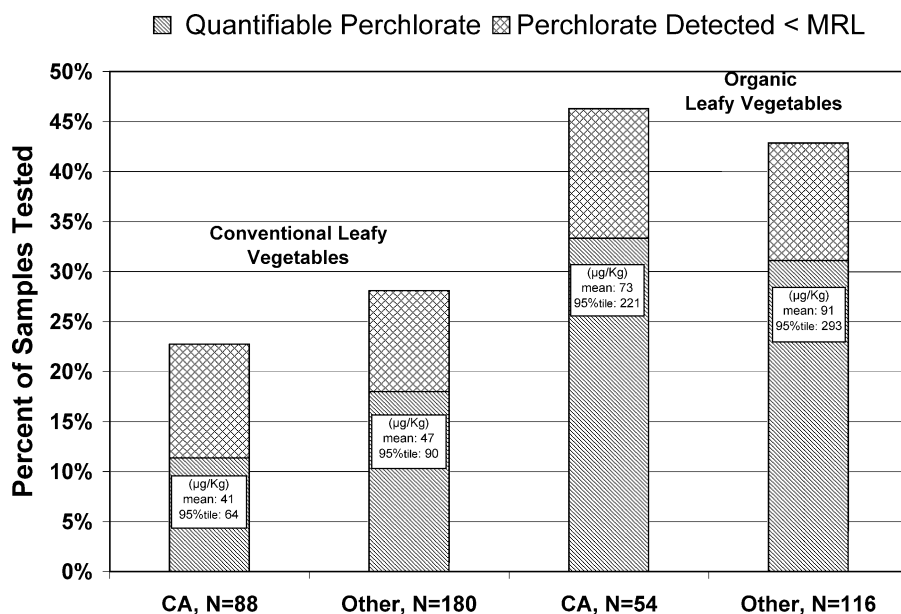


FIGURE 1. Comparison of perchlorate contents of conventional and organic leafy vegetables from California Central and coastal valleys with conventional and organic samples from all of the other regions in the survey combined. The mean and 95th percentile (μg perchlorate/kg wet weight) for the quantifiable samples are inset.

TABLE 4. Hypothetical Mean and 95th Percentile Perchlorate Exposure of Children and Adults Whom Consume Conventional and Organically Produced Leafy Vegetables Calculated from Data in National Survey

consumer age:	2–5		6–11		12–19		20–39		40–59		60 and older	
gender:	boys & girls	boys & girls	males	females	males	females	males	females	males	females	males	females
lettuce ^a	34	41	56	52	63	58	56	59	48	46		
consumption (g/day):												
body weight ^b (kg bw):	16	29	58	53	77	61	78	65	74	65		
Conventional												
exposure ^c ($\mu\text{g/day}$)	0.56 (1.73)	0.67 (2.10)	0.92 (2.86)	0.85 (2.65)	1.0 (3.21)	0.95 (2.96)	0.92 (2.86)	0.97 (3.01)	0.79 (2.45)	0.75 (2.35)		
dose ^d ($\mu\text{g/kg bw}$)	0.03 (0.11)	0.02 (0.07)	0.02 (0.05)	0.02 (0.05)	0.01 (0.04)	0.02 (0.05)	0.01 (0.04)	0.01 (0.05)	0.01 (0.03)	0.01 (0.04)		
Organic												
exposure ($\mu\text{g/day}$)	1.18 (3.71)	1.42 (4.47)	1.94 (6.11)	1.80 (5.67)	2.19 (6.87)	2.01 (6.33)	1.94 (6.11)	2.05 (6.44)	1.67 (5.22)	1.60 (5.02)		
dose ($\mu\text{g/kg bw}$)	0.07 (0.23)	0.05 (0.15)	0.03 (0.11)	0.03 (0.11)	0.03 (0.09)	0.03 (0.09)	0.02 (0.08)	0.03 (0.10)	0.02 (0.07)	0.02 (0.08)		

^a Ref 30. ^b Ref 41. ^c Average of 268 samples for conventional and 170 samples for organic. Estimate includes ND = 10 $\mu\text{g/kg}$ fw, <MRL = 17.5 $\mu\text{g/kg}$ fw and quantifiable values for all leafy vegetables. Values in parentheses are 95th percentile. ^d Dosage ($\mu\text{g/kg bw-day}$) = (g lettuce/kg bw) \times lettuce perchlorate ($\mu\text{g/g}$). Values in parentheses are 95th percentile.

These studies report relative molar potency estimates of perchlorate to nitrate ranging from 250 to 400. More recently, Tonacchera et al. (21) evaluated the relative potency of perchlorate, thiocyanate, and nitrate in vitro using Chinese hamster ovary cells expressing human NIS, and reported a relative potency of perchlorate to nitrate of 240 on an ingested weight basis. In recent work with lettuce produced in the lower Colorado River region, we reported relative iodide uptake inhibition was 2 orders of magnitude greater due to the nitrate in lettuce compared to corresponding trace levels of perchlorate (18). There were significant differences in the nitrate content among the leafy vegetables, with lettuce species having significantly less nitrate compared other leafy vegetable types (Table 5). Furthermore, leafy vegetables produced under organic culture had significantly ($P < 0.001$) lower nitrate levels compared to conventionally grown product irrespective of state or province (Table 6). Organic producers often use composts, fish emulsion products, and green manure legume crops to meet much of the N demand

of crops. These products more slowly release inorganic N compared to most commercial N fertilizer sources, and it is not surprising that organic-produced leafy vegetables had lower nitrate concentrations.

The iodine uptake inhibiting potential of a mixture of inhibitors can be measured by the perchlorate equivalent concentration (PEC), defined as the sum of the concentrations of the inhibitors present divided by their inhibition potency relative to perchlorate on a weight ingestion basis (21). Since perchlorate is estimated as being 240 times more potent than nitrate in inhibiting iodide uptake (21), the PEC of a food containing perchlorate and nitrate is calculated as the concentration of perchlorate plus 1/240 of the concentration of nitrate. Table 7 shows the PEC calculated using the average concentrations of perchlorate and nitrate in our samples. This table shows that the inhibition potential from nitrate was more than 100 times greater than that from perchlorate. Interestingly, the combined perchlorate equivalent concentrations (PEC) of perchlorate and nitrate were greater for

TABLE 5. Types and Number of Leafy Vegetables or Sprouts Sampled, and Mean and Range of Nitrate Concentrations (mg/kg) Observed Nationally

leafy crop	samples collected	mean	min.	max.
iceberg lettuce	63	1288	249	4380
romaine lettuce	82	1356	199	3983
green leaf lettuce	67	1182	193	3034
red leaf lettuce	67	1496	179	5691
butter head lettuce	45	1889	360	5961
baby mix ^a	43	1551	183	4885
endive	9	1141	221	2182
escarole	13	1880	356	6508
arugula	8	3684	1368	4592
spinach	10	2936	772	7034
basil	1	2195	2195	2195
dandelions	4	3302	959	4190
sunflower sprouts	2	515	449	580
kale	7	2956	800	5671
mustard greens	4	2532	770	4678
beet tops	2	2852	2700	3003
mibuna	1	828	828	828
tatsoi	1	4240	4240	4240
chards	1	3622	3622	3622
collards	1	4044	4044	4044
parsely	1	4848	4848	4848

^a Certain blends of baby mix sometimes contain some spinach, dandelions, flowers, or some leafy *Brassica*.

TABLE 6. Summary of Nitrate Contents in Leafy Vegetables by State and Culture

state	total number	median (mg/kg fw)	mean (mg/kg fw)	range (mg/kg fw)
Conventional				
California	88	1488	1548	604–5110
Colorado	6	1717	1672	1144–2106
New Jersey	38	2637	3014	531–7034
New Mexico	9	918	950	249–1661
New York	29	901	1029	221–4059
Michigan	35	1525	1704	282–5671
Ohio	43	1045	1624	366–4848
Quebec	19	1155	1808	269–4844
Organic				
California	54	1462	1762	239–4190
Colorado	13	608	603	344–938
New Jersey	11	979	974	325–1847
New Mexico	16	710	1163	183–4581
New York	30	541	1022	179–4217
Michigan	20	1387	1845	322–4885
Ohio	16	1072	1115	348–2098
Quebec	5	1324	1264	883–1548

TABLE 7. Iodide Uptake Inhibition Potential to Perchlorate and Nitrate in Leafy Vegetables Produced under Conventional and Organic Production Systems

culture	perchlorate (μg/kg fw)	nitrate (μg/kg fw)	PEC ^a of nitrate (μg/kg fw)	total PEC (μg/kg fw)
conventional	16.4	1.724×10^6	7182	7198
organic	34.7	1.356×10^6	5833	5685

^a PEC = $[\text{ClO}_4] + [\text{NO}_3]/240$.

conventionally produced leafy vegetables owing to the significantly greater nitrate concentrations (Table 7). It should be noted that many of the leafy vegetables we sampled were *Brassica* sp. which also contain glucosinolates that release isothiocyanates and thiocyanates upon ingestion. Therefore, total PEC estimates for some of the leafy vegetables represent

under-estimates because their thiocyanate content was not measured or accounted for. Unfortunately, at present we have no method in our laboratory to quantitatively estimate this contribution to total PEC but this should be a focus of future research.

This evaluation supports previous findings with lettuce, human breast milk, dairy milk, bottled water, and human urine, and shows that perchlorate is fairly widespread at low levels in the United States. The evaluation shows higher perchlorate levels in organically produced leafy vegetables compared to conventionally produced vegetables. This work is also consistent with previous work in the lower Colorado River region (18) and suggests that, regardless of culture or region, potential perchlorate exposures from lettuce and other leafy vegetables is negligible relative to acute or long-term harmful amounts.

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