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Perchlorate Accumulation in Forage and Edible Vegetation

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The accumulation of perchlorate in vegetation is becoming a concern, with increasing numbers of sites reporting the presence of perchlorate in groundwater and surface water. This study investigated potential perchlorate uptake and distribution by a variety of forage and edible crops in both the laboratory and the field. Perchlorate concentrations in soybean leaves grown in the greenhouse were significantly higher than perchlorate concentrations in soybean seeds and pods. Perchlorate concentrations in alfalfa grown in sand were significantly lower than those in alfalfa grown in soil. The concentration of perchlorate in tomato was lower in the fruit than the leaves. Commercially grown wheat and alfalfa samples all contained perchlorate, 0.72−8.6 mg/kg of fresh weight (FW) in the wheat stems, 0.71−4.4 mg/kg of FW in the wheat heads, and 2.9 mg/kg of FW in alfalfa. All field garden samples tested (including cucumber, cantaloupe, and tomato) that were irrigated with perchlorate-tainted water contained perchlorate at various concentrations ranging from 0.040 to 1.65 mg/kg of FW. Bioconcentration factors (BCF), ratios of plant fresh weight concentrations to estimated or measured groundwater concentrations [(μg/kg of FW)/μg/L], were all in the same order of magnitude ranging from 215 $\pm$ 126 for wheat stems to 233 $\pm$ 264 for wheat heads and to 380 $\pm$ 89 for alfalfa. BCF for garden fruit samples were much lower (0.5−20). Results from this study highlight the potential for perchlorate exposure by routes other than drinking water.

KEYWORDS: Perchlorate; vegetation; crop; plant; forage; irrigation water; uptake

INTRODUCTION

Perchlorate (ClO_4^-) occurrence in surface water and groundwater has become an ever-increasing concern in the United States since the late 1990s. Perchlorate occurrence is widely distributed in the United States, including perchlorate manufacturer and user facilities in over 44 states and confirmed releases in at least 20 states (*1*). Perchlorate was included on the Contaminant Candidate List (CCL) in 1998 (*2*), and a draft reference dose was issued in early 2003. Currently, some states are adopting action levels for perchlorate in drinking water varying from 1 to 18 μg/L. One issue that has been largely neglected has been the potential exposure of animals and humans to perchlorate through ingestion of plants containing perchlorate. In humans and animals, perchlorate affects thyroid hormone levels by competitively inhibiting the uptake of iodide (*1*, *3*).

There is a large pool of information on the uptake of perchlorate by nonedible aquatic and terrestrial plants, including parrot-feather, smartweed, pickleweed, sweet gum, water lily, and black willow (*4*, *5*, *9*); salt cedar in the Las Vegas Wash (*6*); tobacco plants (*7*); bullrush, crabgrass, cupgrass, and goldenrod (*8*); smartweed, watercress, ash, china-berry, Chinese elm, common paper mulberry, and sugar hackberry. These studies generally suggest that perchlorate is taken up and accumulated mainly in the above-ground portion of the plants, especially in the foliage. Perchlorate concentrations in leaf tissues were 1−2 orders of magnitude higher than perchlorate concentrations in available water, implying a concentration effect in the foliage. In addition, the phytotransformation from perchlorate to innocuous chloride in plant tissues occurs fairly slowly (*10*, *11*). However, few studies have examined the uptake of perchlorate in edible plants, and almost no studies exist on perchlorate distribution within plants (leaves/stems/fruit/roots).

Perchlorate-tainted Colorado River water (*12*) is currently irrigating >70% of the nation's lettuce grown in the winter season, from October to March. A study initiated by the Environmental Working Group, a nonprofit organization, has identified 18% of lettuce samples of a total of 22 purchased in supermarkets as containing detectable perchlorate (*13*). The average amount of perchlorate in the contaminated samples was 70 ppb on the basis of fresh weight (FW). Hutchinson et al. have also observed the accumulation of perchlorate in lettuce irrigated with perchlorate-contaminated water (*14*). In addition to lettuce, perchlorate was also readily taken up in cucumber (*Cucumis sativus* L.) and soybean (*Glycine max*) leaves in a recent laboratory study (*15*). In general, perchlorate is not expected to accumulate in phloem-fed plant tissue, but some

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studies have shown high perchlorate concentrations in berries and grass seeds (*8*).

The primary objective of this study was to investigate potential perchlorate uptake and distribution in a variety of species of food and forage crops in both the laboratory and the field. Experiments conducted included a greenhouse study using tomato, alfalfa,and soybean plants in soil and sand irrigated with perchlorate-contaminated water and a survey of various edible plants and forage crops grown with irrigation water containing perchlorate in Kansas and the Southern High Plains of West Texas.

MATERIALS AND METHODS

Greenhouse Study. Alfalfa, soybeans, and tomatoes were evaluated for perchlorate uptake. Plants were grown in 8-L containers filled with either sand or soil (sandy loam from Terry County, Texas), respectively. Soybeans and alfalfa were grown in sand and soil, whereas tomatoes were grown in sand only. Plants were watered as needed (generally daily) with a solution of 50 ppb (μg/L) of ClO_4^- and 250 mg/L of Peters Professional plant food. Plants were grown until mature, which was defined as (1) flower bloom for alfalfa, (2) pod development for soybeans, and (3) reddening of fruit for tomatoes. At harvest, plants were gently rinsed with deionized (DI) water, weighed, and stored until processing.

Plant samples were processed individually. Soybean plants were divided into leaves, pods, and beans. Alfalfa was separated into "above-ground" and "below-ground" tissue, but only the above-ground tissue was extracted. Tomatoes were divided into leaves and fruits. Plant samples were homogenized, and 0.5-g subsamples were extracted in 30 mL of DI water by boiling for 1 h. Cleanup of samples and analysis followed the general procedure outlined below.

Field Studies. Field samples were collected from Gaines County, Texas, in the Southern High Plains, a region of intense center-pivot-irrigated farming, and from Morris County, Kansas. The Gaines County sites included both commercial fields and home gardens. Winter wheat was collected from four separate fields, and alfalfa was collected from one field. Groundwater in Gaines County is affected by an unknown source of perchlorate countywide. Concentrations of perchlorate in groundwater range from 0.5 to 30 ppb. In addition, two home gardens were sampled for cucumber. Irrigation water was collected from each garden. Cantaloupe, cucumber, and tomatoes were also sampled from a garden in Morris County, Kansas, near a slurry explosives site. Garden irrigation water from this site was also sampled and evaluated for perchlorate. All plants were harvested at apparent maturity.

Plant Preparation, Extraction, and Cleanup. *Wheat and Alfalfa.* Approximately half of the total (0.5 kg) alfalfa or wheat sample from each field was processed. Wheat samples were first separated into heads and stems, air-dried, and ground to a fine powder. About 0.5 g of the ground sample was extracted in 30 mL of DI water in a centrifuge tube by mechanical agitation (3 h). The extractions for each plant sample were done in triplicate to assess variability of the extraction procedure. Alfalfa samples were gently washed in DI water, blotted dry, and then processed as above.

In addition, several wheat plants were randomly selected, and the wheat head was collected for analysis. The wheat heads were manually separated into individual grains of wheat. A portion of the wheat grain was further separated into endosperm (white pulpy inner material) and grain coat (green covering). Two samples of endosperm, one sample of grain coat, and one sample of whole seed were extracted and analyzed for perchlorate. About 0.2 g of the endosperm and grain coat was extracted.

Fruits and Vegetables. Plants were rinsed with water and portioned, and the portions were extracted with water using a method described by Anderson and Wu (*16*). Briefly, plants were extracted in 11-mL cells using Milli-Q water (18 MΩ) with a Dionex (Sunnyvale, CA) Accelerated Solvent Extractor (ASE 200) using the following procedure. Cells were heated for 5 min at 100° C, filled with Milli-Q water, and pressurized to 1500 psi. Total extraction time was 15 min. At the completion of the extraction procedure, extract volume was recorded.

Extract Cleanup. Aluminum oxide was used as an adsorbent to remove the dissolved organic molecules and other interfering ions from plant extracts similar to methods described previously (*16*, *17*). The aluminum oxide adsorbent was prewashed 10 times or more with DI water (using at least 1:1 Al_2O_3/DI water) prior to usage. Five grams of aluminum oxide was added to 5 mL of the extract for 48 h (shaking the sample every 2 h). The sample was filtered, and 5 mL of the filtrate was added to 20 mL of DI for unwashed samples and 45 mL for washed samples.

Analysis. Analysis of perchlorate ion was conducted using a Dionex DX-500 ion chromatography system (Dionex Corp., Sunnyvale, CA) equipped with a GP50 pump, a CD20 conductivity detector, and an AS40 automated sampler. Peaknet was used to control the system. Ion separation was made with a Dionex IonPac AS16 (4 mm) analytical column. The flow rate of eluent, 100 mM sodium hydroxide, was 1.0 mL/min. The injection loop volume was 1000 μL, and the run time for perchlorate analysis was 12 min. An anion self-regenerating suppressor was used for suppressed conductivity detection. An eight-point standard curve was constructed for calibration standards of 2.5, 5, 10, 20, 50, 100, 200, and 500 ppb (μg/L). Computer-generated peak areas were used to determine perchlorate concentrations. Using the method described above, the detection limit for perchlorate in water was 1 μg/L.

Statistical Analysis. Analytical values below detection limits were assigned a value of 0.00 for calculations of means and statistical analysis. Assumptions of homogeneous variances were checked using Bartlett's test. Differences in perchlorate uptake (in various tissues) from soil versus sand were assessed using *t* tests. Differences in contaminant concentrations among plant tissues collected from different fields were evaluated using analysis of variance techniques followed by the Tukey−Kramer Honest Significant Difference means separation test when differences were apparent. Comparisons of mean perchlorate uptake in leaves, pods, and seeds were made using standard analysis of variance techniques incorporating the pot (1, 2, or 3) from which samples were collected as a blocking variable (to minimize the potential influence of the pot on the data set). All statistical tests were considered to be significant when $P \leq 0.05$.

RESULTS

Greenhouse Study. A greenhouse study was conducted to examine the plant accumulation of perchlorate from sand and soil systems under controlled conditions. All plants were watered with a dilute fertilizer solution containing 50 μg/L perchlorate. Soybean leaves, pods, and beans; tomato fruits and leaves; and alfalfa above-ground biomass were analyzed for perchlorate (**Table 1**).

Soybean plants were grown for 12 weeks in both sand and soil (**Figure 1**). Perchlorate concentrations in soybean leaves grown in sand and soil were similar [31 and 26 mg/kg of fresh weight (FW), respectively]. Soybean pod concentrations were similar in plants grown in sand and soil (7.6 ± 3.3 and 2 ± 1.9 mg/kg of FW, respectively). However, pod concentrations were significantly ($F = 15.8$, DF = 4, $P = 0.017$, where F is the F statistic and DF is the degrees of freedom, respectively) higher in plants grown in sand compared to those grown in soil (0.6 ± 0.05 and 0.07 ± 0.13 mg/kg of FW, respectively). Due to the low concentrations, soybean seed samples were confirmed using LC-MS. Perchlorate concentrations were significantly higher in soybean leaf tissues than in pod or seed tissues for both sand and soil ($F = 133$, DF = 8, $P = 0.0002$, and $F = 33.5$, DF = 8, $P = 0.0032$, respectively). There were no significant differences between pod and seed tissue concentrations for plants grown in sand or soil.

Perchlorate concentrations in sand-grown alfalfa were significantly lower ($F = 26.3$, DF = 5, $P = 0.007$) than in soil-grown alfalfa (8.7 ± 1.0 and 19 ± 2.2 mg/kg of FW, respectively). These concentrations were also lower than in the

Table 1. Accumulation of Perchlorate in Plants Grown in a Greenhouse in either Sand or Soil

plant	tissue	pot	no. of plants per pot	growth period (weeks)	% water in plant tissue		tissue concn (mg/kg of DW)		av-dry (mg/kg of DW)		av-fresh (mg/kg of FW)		BCF (FW basis)	
					sand	soil	soil	sand	soil	sand	soil	sand	soil	sand
soybean	leaves	1	2	12	44 (5.8)	53 (6.8)	39 (7.8)[a]	49 (13)	55 (17)[b]	55 (5.2)	26 (8.0)	31 (2.9)	520 (160)	620 (58)
		2					52 (0.0)	57 (5)						
		3					73 (14)	59 (11)						
	pod	1	2	12	24 (3.6)	40 (4.2)	1.1 (0.07)	13 (1.1)	3.3 (3.2)	10 (4.3)	2 (1.9)	7.6 (3.3)	40 (38)	150 (66)
		2					1.9 (0.42)	5.6 (1.9)						
		3					7.5 (0.78)	11 (1.0)						
	seeds	1	2	12	33 (20)	57 (15)	ND[c]	0.9 (0.6)	0.17 (0.31)	0.91 (0.08)	0.07 (0.13)	0.6 (0.05)	1.4 (2.6)	12 (1)
		2					ND	0.9 (0.6)						
		3					0.6 (0.44)	1.0 (0.6)						
tomato	leaves	1	1	12	70 (4.1)	NS[d]	NS	37 (4.4)	NS	36 (1.3)	NS	11 (0.91)	NS	220 (18)
		2						35 (20)						
		3						35 (3.0)						
	fruit	1	1	12	81.4 (0.2)	NS	NS	0.8	NS	0.9 (0.1)	NS	0.18 (0.02)	NS	3.4 (0.4)
		2						1.0						
		3						NS						
alfalfa	above-ground biomass	1	>10	8	53 (4.6)	42 (0.8)	33 (6.7)	18 (1.4)	32 (3.8)	18.5 (2.3)	19 (2.2)	8.7 (1.0)	360 (44)	170 (20)
		2					36 (6.7)	21 (2.8)						
		3					28 (1.4)	17 (5.0)						

[a] Average (standard deviation) based on multiple samples from each pot. [b] Average (standard deviation) based on samples from all pots. [c] Below detection. [d] Not sampled.

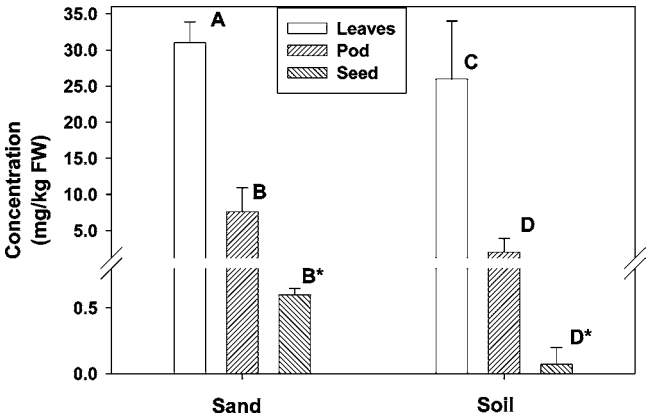

Figure 1. Distribution of perchlorate in soybean plants grown in sand and soil. Different letters indicate significant differences between soybean compartment perchlorate concentrations within a treatment (sand or soil); * indicates a significant difference between treatments (sand versus soil) for a given compartment.

soybean leaves and tomato leaves. At least two reasons exist for the lower alfalfa concentrations: (1) alfalfa plants were grown for only 8 weeks (lower exposure) and (2) both stem and leaf were combined for alfalfa, whereas stems were excluded from soybean and tomato extractions. Perchlorate accumulation has been shown to be greatest in actively transpiring tissue, and past studies have shown significantly lower concentrations in stems compared to leaves (*15*).

Two tomato plants bore one fruit each. The concentration of perchlorate was lower in the fruit (0.18 ± 0.02 mg/kg of FW) than in the leaves and was very similar to the concentration in soybean seeds. Perchlorate in tomato fruit samples was confirmed using LC-MS.

Field Studies. Plant samples were collected from both commercial agricultural operations and home gardens. Commercial crops sampled included winter wheat and alfalfa collected in the Southern High Plains of Texas. Wheat samples were sectioned into stems and heads, whereas alfalfa samples included all above-ground biomass. Perchlorate was found in all samples tested, ranging from 0.72 to 8.6 mg/kg of FW and from 0.71 to 4.4 mg/kg of FW in the stems and heads, respectively, of wheat samples; 2.9 mg/kg of FW was found in alfalfa (**Table 2**). In three of four fields tested perchlorate concentrations in wheat stems were higher (~1−4-fold) than in wheat heads. In the one field in which perchlorate concentrations were higher in wheat heads, the variation in the concentration for perchlorate in the wheat head was extremely large (~2 times the average). Sample sizes for garden vegetables and fruits were quite small. All samples tested contained perchlorate at various concentrations ranging from 0.040 to 1.65 mg/kg on a fresh weight basis (**Table 2**).

Commercial samples were collected in an area that uses extensive center-pivot irrigation. Samples from two fields were tested for the possibility that perchlorate was present as a dried residue salt on the outside of the plant samples. Samples were gently rinsed in DDI water before extraction. No significant difference was found between concentrations in tissue before and after washing. Although some perchlorate may be present as a dried residue, most of the perchlorate is likely to be present within the plant tissue. To further determine the extent of partitioning within the wheat head, seed coat, endosperm, and whole seed samples from field 1 were analyzed for perchlorate. Whole head samples from this field averaged 0.71 mg/kg of FW, whereas the whole grain (not including the chaff), grain coat, and endosperm had measured concentrations of 1.3, 3.3, and ND mg/kg of FW, respectively. It should be noted that these extractions were not done in replicate due to the difficulty of separating the samples and are mainly meant to highlight the relative distribution of perchlorate.

Irrigation water could not be sampled at the time of collection; however, groundwater in these counties has been extensively sampled, and estimates of groundwater concentrations were determined on the basis of perchlorate distribution maps of this area (**Table 2**). In general, perchlorate concentrations in wheat stems were significantly ($F = 203$, DF = 11, $P < 0.0001$) higher in plants grown on irrigation water with higher perchlorate concentrations (**Figure 2**) but not in wheat heads, probably due to the larger amount of variation. Bioconcentration factors (BCF), ratios of plant fresh weight concentrations to estimated groundwater concentrations [(μg/kg of FW)/μg/L], were all in the same order of magnitude ranging from 103 to 390 (average $= 215 \pm 126$) for wheat stems, from 78 to 628 (average = 233

Table 2. Accumulation of Perchlorate in Plants Collected from Field Sites

location	plant	*N*	plant concn (mg/kg) DW	plant concn (mg/kg) FW	plant concn (mg/kg) washed DW	irrigation water concn (μg/L)	BCF (plant FW concn/water concn)
Gaines County, Texas							
field 1	wheat stem	3	4.1 (1.3)	1.9 (0.61)	1.5 (0.89)	9[b]	211 (68)
	wheat head	3	1.5 (2.0)	0.71 (0.20)[a]	2.8 (0.83)		78 (102)
field 2	wheat stem	3	1.8 (1.1)	0.94 (0.60)	NA	6[b]	157 (100)
	wheat head	3	1.5 (0.42)	0.79 (0.23)	NA		132 (38)
field 3	wheat stem	3	1.6 (0.42)	0.72 (0.19)	1.1 (0.60)	7[b]	103 (21)
	wheat head	3	9.5 (15)	4.4 (7.0)	1.4 (0.90)		628 (1,000)
field 4	wheat stem	3	17 (0.36)	8.6 (0.19)	NA	22[b]	390 (8.6)
	wheat head	3	4.0 (1.2)	2.1 (0.65)	NA		95.4 (30)
field 5	alfalfa	3	5.2 (0.89)	2.9 (0.49)	NA	5.5[b]	380 (89)
garden 1	cucumber (whole fruit)	1	NA[c]	0.04	NA	20.7	1.9
Morris County, Kansas							
garden	cucumber (whole fruit)	1	NA	0.77	NA	81 (1.4)	9.4
	cantaloupe (flesh)	1	NA	1.6	NA		20
	tomato (whole fruit)	1	NA	0.22	NA		2.7
	tomato (whole fruit)	1	NA	0.042	NA		0.5

[a] Average (standard deviation) of multiple samples collected from each field. [b] Estimated irrigation water concentration based on perchlorate distribution produced from nearby wells. [c] Not available.

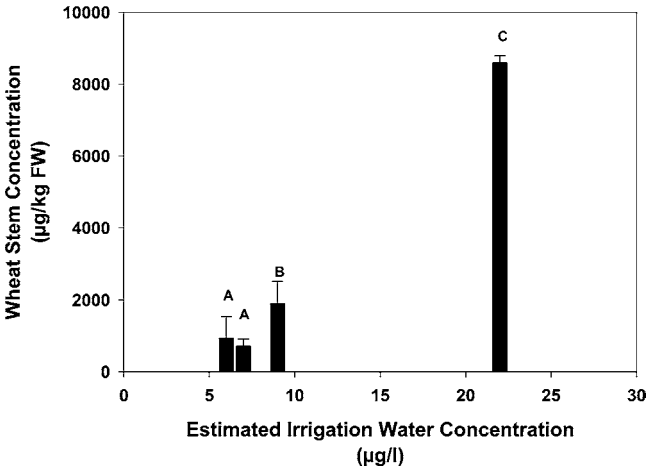

Figure 2. Relationship between predicted irrigation water perchlorate concentration and wheat stem perchlorate concentration. Columns with different letters indicate significant differences in perchlorate concentrations.

Table 3. Bioconcentration Factors (BCF) of All Plants Form Field and Greenhouse Studies Expressed on a Fresh Weight Basis

plant type	BCF (field)	BCF (greenhouse) soil	BCF (greenhouse) sand
alfalfa	380 (89)[a]	360 (44)	170 (20)
wheat stem	215 (126)[b]	NS	NS
wheat head	233 (264)[b]	NS	NS
soybean leaf	NS[c]	520 (160)	620 (58)
soybean pod	NS	40 (38)	150 (66)
soybean seed	NS	1.4 (2.6)	12 (1)
tomato leaf	NS	NS	220 (18)
tomato fruit	1.6 (1.55)[b]	NS	3.4 (0.4)
cucumber fruit	5.7 (5.3)[b]	NS	NS
cantaloupe fruit	20	NS	NS

[a] Average (standard deviation) based on multiple samples from same location. [b] Average (standard deviation) based on samples from different locations. [c] Not sampled.

$\pm$ 264) for wheat heads, and 380 $\pm$ 89 for alfalfa (**Table 3**). Garden fruit samples from the Kansas site were watered with a higher perchlorate concentration in water (81 μg/L) but, even so, ratios of plant to water concentrations (BCF) were much lower (0.5−20) (**Table 2**), supporting the importance of transpiring tissue in perchlorate accumulation.

DISCUSSION

Both laboratory and field data collected in the present study support the hypothesis that perchlorate accumulates in food and forage crops when irrigated with perchlorate-contaminated water. Experiments were performed during this study both to evaluate the potential for perchlorate uptake in edible plants when exposed to perchlorate and to test vegetation in areas with perchlorate-contaminated groundwater. Laboratory studies and field sampling indicated that perchlorate is readily accumulated in crops. Common vegetable crops were included in the perchlorate uptake experiments performed in the laboratory. All were found to take up perchlorate when exposed, with the highest exposure likely to come from green leafy vegetation.

Previous studies indicated that a variety of noncrop plants collected from the field were capable of perchlorate uptake if exposed to perchlorate (*6*, *9*). Perchlorate concentrations in terrestrial plants were more variable than in aquatic plants, probably due to the more variable nature of the source water. Most plant species obtain their water from within the vadose zone of the soil profile and not from bulk free-flowing surface water. As such, changes in biogeochemistry throughout the year coupled with fluctuating water tables and water availability equate to a highly variable source contribution. In addition, potential leaching of perchlorate from leafy vegetation during precipitation events may also contribute to the large variability found. Regardless, leaf concentrations of perchlorate generally increased throughout the growing season and in general represented a higher perchlorate burden by mass than source water. Vegetative uptake of perchlorate in terrestrial species appears to be a function of both exposure concentration and length of exposure (*9*).

Results from this study have shown a high bioconcentration effect (BCF ranged from 40 to 628) of perchlorate in the transpiration tissues of food crops, such as soybean leaves and pods, tomato leaves, alfalfa, and wheat stems and heads. Perchlorate accumulation was also detected in edible portions (fruits and seeds) of several garden plants, although with a lower bioconcentration (BCF ranged from 0.5 to 20). In general, results from our crop study were consistent with the previous findings on noncrop terrestrial plants. Another study (*9*) indicated that perchlorate was selectively partitioned in china-berry and

mulberry trees, with leaf concentration of 1.3−5.0 mg/kg of dry weight (DW) and fruit concentration of 0−0.5 mg/kg of DW.

If plant species are irrigated naturally or artificially with water containing perchlorate, uptake will occur, including uptake into edible portions of the plant. Humans may also be exposed to perchlorate indirectly via consumption of plants that have taken up this contaminant. A recent study found perchlorate in cow's milk (*18*), implying the possible trophic transfer from plants to animals. Our findings, supported from other earlier perchlorate uptake data in leafy food crops (*13*, *15*), suggested that perchlorate accumulation in food crops could present another human exposure route to perchlorate through trophic transfer in food chains in addition to drinking water. The perchlorate uptake and accumulation in leafy crops or edible parts of other food crops may present a health hazard to humans and animals.

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