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Potential perchlorate exposure from *Citrus* sp. irrigated with contaminated water

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Abstract

Citrus produced in the southwestern United States is often irrigated with perchlorate-contaminated water. This irrigation water includes Colorado River water which is contaminated with perchlorate from a manufacturing plant previously located near the Las Vegas Wash, and ground water from wells in Riverside and San Bernardino counties of California which are affected by a perchlorate plume associated with an aerospace facility once located near Redlands, California. Studies were conducted to evaluate the uptake and distribution of perchlorate in citrus irrigated with contaminated water, and estimate potential human exposure to perchlorate from the various citrus types including lemon (*Citrus limon*), grapefruit (*Citrus paradise*), and orange (*Citrus sinensis*) produced in the region. Perchlorate concentrations ranged from less than 2–9 μg/L for Colorado River water and from below detection to approximately 18 μg/L for water samples from wells used to irrigate citrus. Destructive sampling of lemon trees produced with Colorado River water show perchlorate concentrations larger in the leaves (1835 μg/kg dry weight (dw)) followed by the fruit (128 μg/kg dw). Mean perchlorate concentrations in roots, trunk, and branches were all less than 30 μg/kg dw. Fruit pulp analyzed in the survey show perchlorate concentrations ranged from below detection limit to 38 μg/kg fresh weight (fw), and were related to the perchlorate concentration of irrigation water. Mean hypothetical exposures (μg/person/day) of children and adults from lemons (0.005 and 0.009), grapefruit (0.03 and 0.24), and oranges (0.51 and 1.20) were estimated. These data show that potential perchlorate exposures from citrus in the southwestern United States are negligible relative to the reference dose recommended by the National Academy of Sciences.

Keywords: Lemon (*Citrus limon*); Grapefruit (*Citrus paradise*); Orange (*Citrus sinensis*); Colorado River; Perchlorate

1. Introduction

Perchlorate has been discovered in surface and ground water 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 has been detected in several non-crop plant species in non-cultivated ecosystems exposed to aerospace and defense-related perchlorate contamination [2–5]. Accumulation of perchlorate in tobacco [6] fertilized with perchlorate-containing Chilean nitrate [7,8] is also documented. A number of studies have shown perchlorate accumulation in edible leafy vegetables irrigated with perchlorate-contaminated water [9–11]. Data also indicate potential perchlorate accumulation in fruiting and seed crops irrigated with contaminated water but bioconcentration appears lower compared to leafy vegetation [12].

A substantial area of citrus is irrigated with perchlorate-contaminated water in the southwestern United States. Citrus produced in the lower Colorado River valleys of Arizona and California and the Coachella Valley of California are irrigated with Colorado River water, which has had perchlorate concentrations ranging from 5 to 9 μg/L [13]. Approximately 5 billion m^3 of water are diverted at the Imperial Diversion Dam to irrigated crops in southwestern Arizona and southern California. Perchlorate contamination in the Colorado River is introduced into Lake Mead by a perchlorate salt manufacturing plant previously located near the Las Vegas Wash.

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Citrus produced in portions of Riverside and San Bernardino counties of California outside the low desert are irrigated with wells affected by a perchlorate ground water plume associated with an aerospace facility near Redlands, California. The objectives of this study were to evaluate the uptake and distribution of perchlorate in *Citrus* sp. irrigated with contaminated water, and estimate potential human exposure to perchlorate from the various citrus types produced in the region.

2. Experimental

2.1. Uptake and distribution

These samples were actually generated from another study aimed at evaluating the redistribution of ^{15}N-labeled nitrogen in young citrus. Nine five-year-old lemon "Limoneira 8A Lisbon" on "Volkamariana" rootstock at the Yuma Mesa Agricultural Center were sacrificed for these evaluations. These trees were destructively sampled December 5, 2001. All leaves and fruit were hand harvested from each tree. The branches were then removed with a saw from the trunk of the tree. The whole fruit (peel and pulp) was cut into wedges and the branches were cut further into small segments. All leaves, fruit wedges, and branch segments were labeled appropriately, and placed in an oven for drying. The stumps and roots of each tree were pulled out of the ground with a tractor and chain, labeled, and transported to an open storage area for air-drying.

The leaves and fruit wedges were ground directly after drying. The branch segments were ground after processing through a wood chipper. Following 4 months of air-drying, the trunks and roots were separated and processed for grinding. Because trunk segments caused the mechanical failure of two wood chippers in rapid succession, we improvised another approach for processing the trunk and root. Trunk and roots were cut at short intervals (approximately 5 cm) with a chain saw and wood shavings were collected and composited for each tree, and dried in an oven. This composite sample was ground for analysis.

3. Survey of fruit and leaves

Citrus samples were collected during harvest season from fields across southwestern Arizona and southern California during 2004–2005. Samples were collected from different types of citrus including lemon (*Citrus limon*), grapefruit (*Citrus paradise*), and orange (*Citrus sinensis*). The number and location of samples were reflective of the commercial industry. The majority of citrus produced in the lower Colorado River valleys are lemons, with modest orange production, and no commercial grapefruit products. All lemon samples, and a few orange samples, were collected in this area. The only grapefruit collected in this area was from the University of Arizona Research Farm near Yuma, Arizona. Most of the citrus produced in the Coachella Valley, and in the higher altitude regions of southern California, are oranges with modest grapefruit production. It was from this area we collected most orange and grapefruit samples. Lemon, orange, and grapefruit samples were also collected from an orchard in Los Angeles County, suspected of being irrigated with water affected by a perchlorate plume. For each sample we attempted to collect 10 fruits at random from each orchard. For a subset of these we collected corresponding leaf samples from the trees. For all fruit samples, peel and pulp were separated by hand and the leaves, peel, and pulp were frozen separately. The frozen samples were freeze-dried on a Labconco freeze drier. Freeze-drying of leaf and peel tissue typically was complete within 48 h but pulp tissue often required 96 h. Weights before and after freeze-drying were recorded and the samples were subsequently ground and stored in vials for extraction.

3.1. Extraction of perchlorate from plant material

We used an extraction procedure described previously [14] 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 Gelman ion membrane syringe filters. Two milliliter of the above extract (extract 1) was reacted 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 Gelman 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). Before loading on the IC-CD, the extracts were allowed to reach room temperature and were filtered through pre-conditioned 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 used for IC-CD analysis.

3.2. Perchlorate analysis

Perchlorate analyses were initially performed by IC-CD using a Dionex 2500 described previously [11]. Briefly, this unit consists 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 an LC 30 chromatography oven. We used 50 mM KOH eluent and 50 mM sulfuric acid suppression. A minimum of 10% of the samples were extracted with a 100 μg/L perchlorate standard to yield 10 μg/L perchlorate standard addition after dilution. The method detection limit (MDL) was determined using the procedure outlined in EPA method 314.0 [15] using seven replicates of a standard in reagent water. The calculated MDL was 0.2 μg/L using a 0.5 μg/L standard. We set the minimum reporting level (MRL) for citrus plant extracts at 1.5 μg/L. As a standard practice we ran 10% duplicate extractions in addition to the 10% spiked additions. Duplicate aliquots of a given extraction were always analyzed. We generally repeated analysis if recovery of standards and standard additions was less than 85% and variation among duplicates exceeded 25%.

Branch, trunk, and fruit tissue were below detection by IC-CD and root tissue gave false positive perchlorate peaks by IC-CD. Accurate quantification of these tissues required IC/MS/MS. Perchlorate concentrations measured in leaves by IC-CD and IC/MS/MS agreed closely but a few leaf extracts produced co-eluting peaks making accurate integration difficult. Leaf sample extracts with problematic matrices, those with co-eluting peaks, and several samples at random were sent out for IC/MS/MS analysis. Therefore, all root, trunk, branch and fruit tissues from the destructive sampling study, all fruit pulp from the survey, a selected subset of peel samples from the survey, and approximately 25% of all leaf samples collected, were sent to a laboratory for analysis by IC/MS/MS using an ^{18}O internal standard methodology similar to that reported by others [16]. Briefly, 0.5 mL of aqueous sample extract was spiked with an isotopically labeled internal standard ($Cl^{18}O_4^-$) and diluted 1:1 with deionized water. This solution was subsequently analyzed using ion chromatography–electrospray ionization–tandem mass spectrometry. Perchlorate was 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 perchlorate reference solutions (AccuStandard, New Haven, CT, USA); 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 0.02 μg/L and the MRL was 0.1 μg/L.

The MRL would be approximately 375 μg/kg dw by IC-CD and 25 μg/kg dw by IC/MS/MS using our extraction ratio. Dry matter content ranged from 33 to 98% for leaves, 14 to 30% for peels, and 8 to 17% for fruit pulp. Therefore, the MRL levels by IC-CD would be approximately 190, 75, and 38 μg/kg fw, for leaves, peel, and pulp, respectively. Reporting levels by IC/MS/MS would be approximately 13, 5, and 2.5 μg/kg fw for leaves, peel, and pulp, respectively.

3.3. Perchlorate concentration in irrigation water

Aliquots of composite Colorado River water samples, collected by the U.S. Bureau of Reclamation (USBOR) at the Imperial Diversion Dam, from March 2003 through September 2005, were analyzed for perchlorate in our laboratory. Water samples from wells and reservoirs used for irrigation were also collected at the time of citrus sampling. These water samples were analyzed for perchlorate using EPA Method 314.0 [15]. We estimated a reporting level of 1 μg/L in water using methods described above. Perchlorate concentrations of Colorado River at the Imperial Dam were compared to samples collected up-stream at Willow Beach by the Nevada Division of Environmental Protection from December 1999 through April 2005 [17].

3.4. Exposure estimates

An MRL of 0.1 μg/L by IC/MS/MS would correspond to approximately 2.5 μg/kg fw for fruit pulp. For values below MRL, we used estimates of 1.25 μg/kg fw and for values below detection we used estimates of 0.625 μg/kg fw. We used median perchlorate concentrations in the edible fruit pulp and mean and 95th percentile consumption estimates [18] to estimate exposures.

4. Results and discussion

Perchlorate concentrations of the Colorado River ranged from 1 to 9 μg/L (Fig. 1). Data were collected by the Nevada Department of Environmental Protection at Willow Beach, 11 miles down stream of Lake Mead, are shown from late 1999 through April 2005. We did not begin collecting data at Imperial Diversion Dam, 290 miles downstream of Lake Mead, until March 2003. There was some temporal variation in perchlorate concentrations between the two sampling locations which is not surprising considering that water travel times, water quantity, and water quality are all potentially altered by diversion dams, storage reservoirs, and tributaries along the river. Nevertheless, the data generally compare favorably where the average concentrations from March 2003 through April 2005 were 4.1 and 4.0 μg/L at Willow Beach and Imperial Diversion, respectively. Thus, where we do not have data for the Imperial Diversion Dam, we used data from Willow Beach as a reasonable estimate of perchlorate concentrations of irrigation water. Studies have shown that perchlorate is not physically or chemically retained by soil [19,20]. Thus, perchlorate is largely transported into and through soils with irrigation water and the perchlorate concentration of this water is the most reliable estimate of plant available perchlorate over a growing season.

The concentrations of perchlorate in other water sources used to irrigate citrus ranged from below detection from well water in Los Angeles County and some reservoirs and wells in the Coachella Valley to18 μg/L from a well in Loma Linda, near

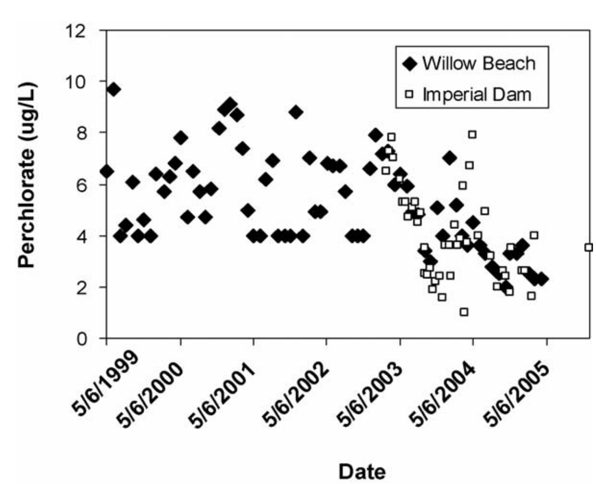

Fig. 1. Perchlorate concentration in Colorado River over study period.

Redlands (Table 1). It should be noted that some citrus in the Coachella Valley is irrigated with surface deliveries from the Colorado River, some citrus is irrigated with ground water, and some is irrigated with both sources. It has been alleged that ground water in the Coachella Valley has been contaminated with perchlorate from recharge from the Colorado River [21] and it is debated whether this is from an intentional recharge program administered by the irrigation district or incidental recharge through agricultural irrigation. Colorado River water transported through the aqueduct has also been used to recharge ground water along its route from the Colorado River, near Parker, to Los Angeles and the river might have contributed toward the perchlorate contamination of other ground water sources used to irrigate citrus. Trace levels of perchlorate were found in the fruit from some orchards in the Coachella Valley where the corresponding water samples tested below detection by IC-CD. It is likely these orchards are irrigated with other sources of water in addition to the water collected at the time of sampling. Furthermore, previous studies have shown perchlorate in rainfall [22] and bottled water [23] at sub part per billion levels and we cannot rule out the presence of perchlorate below our detection by IC-CD. However, for the orchard in Los Angeles County we found no detectable perchlorate in lemon, orange, and grapefruit, where the only source of water was a well where perchlorate was below detection by IC-CD.

We do not consider fertilizer a likely source of perchlorate in the citrus samples collected. As noted previously, the only fertilizer source with a significant perchlorate content is Chilean nitrate [8]. More than one of the authors work closely with citrus producers in the western United States and could identify no situations where Chilean nitrate was used in recent history. A review of the scientific literature show some use of Chilean nitrate in N fertilizer experiments initiated in the 1920s [24,25] but could identify no use in several other fertilizer N experiments conducted from the 1950s through more recent times [26–28]. Some low biuret urea is used for foliar fertilizer of citrus trees [29]. This history suggest that Chilean nitrate was used by some producers decades ago but its use was discontinued as other more economical N fertilizer sources became available through the Haber process. As a result of large leaching fractions of irrigation waters used in the western United States non-reactive anion, such as perchlorate would be expected to leach out of the crop-rooting zone within a season after application [19,20].

Table 2
Perchlorate concentrations of various tree parts for destructively sampled lemon trees

Tree part	Perchlorate (μg/kg dw)[a]	
	Range	Mean
Roots	<DL–55	<MRL
Trunk	<DL–<MRL	<MRL
Branches	<DL–65	26
Leaves	699–4931	1835
Fruit	64–195	128

[a] MRL is minimum reporting level and DL is detection limit.

The average perchlorate concentrations (μg/kg dw) in lemon trees irrigated with Colorado River water are shown in Table 2. Perchlorate in the trunk was below MRL and perchlorate in the roots and branches was close to MRL by IC/MS/MS. Perchlorate concentrations in the fruit (peel and pulp) and leaves were 128 and 1835 g/kg dw, respectively. The trees were 5-years-old and it is estimated they were irrigated with water having an average perchlorate close to 6 μg/L. Water consumption of an individual citrus tree can range from 80 to 100 m^3 annually [30] and citrus retains leaves for 2–3 years [31]. Thus, there is a large potential for perchlorate accumulation in these transpiring leaves through xylem transport where citrus is irrigated with contaminated water.

These data are generally consistent with data collected in the survey, which show much larger accumulations in the leaves compared to the fruit (Tables 3 and 4). The larger variation in concentration in leaves collected in the survey is likely the result of varying perchlorate concentrations of water sources and varying age of leaves sampled. The trees that were destructively sampled were all of the same age, adjacent in the same field, irrigated with the same Colorado River water over the same time interval, and our sample represented a composite of all the leaves on the tree. For the survey we sampled trees of varying age

Table 1
Perchlorate concentration of various water sources used to irrigate citrus

Location	County/state	Date collected	Perchlorate (μg/L)[a]
Coachella Valley	Riverside Co., CA, USA	June 30, 2004	4.1
Loma Linda	San Bernardino Co., CA, USA	December 7, 2004	18.1
Riverside	Riverside Co., CA, USA	January 4, 2005	3.4
Riverside	Riverside Co., CA, USA	February 14, 2005	1.0
Riverside	Riverside Co., CA, USA	February 14, 2005	2.1
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	<DL
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	2.7
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	<DL
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	<DL
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	11.4
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	11.6
Coachella Valley	Riverside Co., CA, USA	February 15, 2005	2.5
Loma Linda	San Bernardino Co., CA, USA	August 20, 2005	15.8
Canoga Park	Los Angeles Co., CA, USA	October 13, 2005	<DL

[a] DL is detection limit.

Table 3
Concentrations of perchlorate in leaves and peel samples collected in survey

Crop	*n*	Dry weight (μg/kg)			Fresh weight (μg/kg)		
		Minimum	Maximum	Mean	Minimum	Maximum	Mean
Leaves							
Lemon	11	567	4979	2357	283	3629	1695
Grapefruit	4	372	4346	1659	145	1738	647
Orange	8	894	8987	2875	430	4494	1424
Peel							
Lemon	5	29	261	115	5	41	18
Grapefruit	4	17	149	80	4	29	17
Orange	12	89	731	199	22	189	48

Table 4
Hypothetical mean and 95th percentile perchlorate exposure of children and adults who consume citrus

Crop	*n*	Perchlorate (μg/kg fw)			Citrus consumption (g/day)		Exposure (μg/day)[b]	
		Range	Mean[a]	Median	Children[a]	Adult[a]	Children[a]	Adult[a]
Lemon	33	<DL–14.8	2.3 (6.1)	1.3	4 (27)	7 (50)	0.005 (0.035)	0.009 (0.065)
Grapefruit	15	<DL–16.2	3.3 (8.1)	1.3	24 (121)	185 (703)	0.03 (0.16)	0.24 (0.91)
Orange	28	<DL–37.6	7.4 (25.3)	4.8	107 (323)	249 (744)	0.51 (1.55)	1.20 (3.57)

[a] Values in parenthesis represent 95th percentile numbers.
[b] Exposure estimates calculated by (median perchlorate content, μg/kg fw) × (mean (or 95th percentile) consumption estimates, kg).

(7–30-years-old), leaves were collected at random from the tree canopy, and we did not distinguish leaf age. The larger values for perchlorate concentration in all tissues are generally associated with the trees sampled at Loma Linda.

Perchlorate concentrations were notably lower in the fruit peel and pulp compared to the leaves (Tables 3 and 4). Concentrations in the fruit pulp ranged from below detection in an orchard in Los Angeles County to 38 μg/kg fw at Loma Linda. Because the initial sample from Loma Linda appeared to be an outlier compared to other samples, we collected additional samples 6 months later, and obtained similar results (water 16 μg/L and fruit pulp 29 μg/kg). Water transpiration through fruit tissue is less than the leaves and a significant portion of the accumulated solutes in the fruit are transported through phloem transport [32]. Although we are inclined to assume much less perchlorate is translocated to the fruit, compared to the leaves, we cannot rule out biochemical reduction of the perchlorate which has been identified as being important in certain plant species [33,34].

Mean hypothetical adult perchlorate exposure in the edible fruit averaged 0.009, 0.23, and 1.20 μg/day for lemons, grapefruit, and oranges, respectively (Table 4). Similar results for children averaged 0.005, 0.03 and 0.51 μg/day. It should be noted that these estimates for oranges include those samples collected at Loma Linda, which is a private orchard and this citrus is not marketed commercially. Estimated dosages for a 70 kg adult [35] from oranges would be 0.02 μg/kg bw which is less than 5% of the no effect reference dose of 0.7 μg/kg recommended by the National Academy of Sciences (NAS). Estimating dosage for children are more difficult because consumption data are limited and our consumption estimate includes a wide range of children's ages and body weights. However, even considering a child with a 10 kg body weight, the estimated dosage would be approximately 10% the NAS-recommended reference dose. The NAS reference dosage is based upon a no-observed effect level of 7 μg/kg from human iodide uptake studies [36] to which a 10-fold uncertainty factor was applied to address all potentially sensitive subpopulations [37].

It is important to note that from previous work with leafy vegetables [11,38] we obtained reasonable estimates of exposure by IC-CD using estimated values below levels of quantification and detection. If we had used a similar approach for citrus and relied on IC-CD analysis only, we would have overestimated perchlorate exposure by a factor of 4. For crops like citrus, where perchlorate accumulation is low but human consumption is high, accurate estimates of exposure require sensitive and selective analytical methodology such as IC–MS/MS.

In conclusion, citrus trees do accumulate perchlorate from low concentrations in irrigation water. There is a potential for high perchlorate concentrations to accumulate in transpiring leaves but only trace levels are found in the edible fruit. These data show that potential perchlorate exposures from citrus in the southwestern United States are small relative to the reference dose recommended by the NAS.

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