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Perchlorate content of plant foliage reflects a wide range of species-dependent accumulation but not ozone-induced biosynthesis☆,☆☆

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ABSTRACT

Perchlorate (ClO_4^-) interferes with uptake of iodide in humans. Emission inventories do not explain observed distributions. Ozone (O_3) is implicated in the natural origin of ClO_4^-, and has increased since pre-industrial times. O_3 produces ClO_4^- *in vitro* from Cl^-, and plant tissues contain Cl^- and redox reactions. We hypothesize that O_3 exposure may induce plant synthesis of ClO_4^-. We exposed contrasting crop species to environmentally relevant O_3 concentrations. In the absence of O_3 exposure, species exhibited a large range of ClO_4^- accumulation but there was no relationship between leaf ClO_4^- and O_3, whether expressed as exposure or cumulative flux (dose). Older, senescing leaves accumulated more ClO_4^- than younger leaves. O_3 exposed vegetation is not a source of environmental ClO_4^-. There was evidence of enhanced ClO_4^- content in the soil surface at the highest O_3 exposure, which could be a significant contributor to environmental ClO_4^-.

1. Introduction

Perchlorate (ClO_4^-) salts are detected across large areas of the environment. The ClO_4^- anion is a tetrahedral cluster of oxygen with a central chlorine, exhibiting similar ionic radius and charge density to iodide. It therefore disrupts thyroid metabolism in mammals, including humans, by interference with uptake of iodide. In plants ClO_4^- competes for uptake with other anions such as NO_3^-.

ClO_4^- is highly soluble in water and does not adsorb substantially to mineral or organic constituents of soils. There appear to be atmospheric sources of naturally occurring ClO_4^-, derived from atmospheric oxidation of Cl^- and other Cl species, potentially mediated by lightning, ultraviolet radiation, or tropospheric O_3 (Michalski et al., 2004; Dasgupta et al., 2006; Rajagopalan et al., 2006; Jaegle et al., 1996; Kang et al., 2006; Rao et al., 2007, 2012). In areas with significant rainfall it is readily leached out of surface strata into ground and surface waters (Urbansky and Brown, 2003). In arid regions it accumulates at the surface and considerable concentrations have been detected (Rao et al., 2007; Jackson et al., 2010). If other areas are equally prolific sources of ClO_4^-, but lack the concentrating mechanism, this would imply widespread, multimedia dispersal of ClO_4^- across the environment. ClO_4^- occurrence at significant concentration in surface waters such as the Colorado River, and in ground waters such as the Ogalalla Aquifer in the Midwestern U.S. emphasize the breadth of the problem (Dasgupta et al., 2006; Rajagopalan et al., 2006).

In addition to natural atmospheric sources, a number of anthropogenic point sources have been identified. These include industrial and military application as a rocket fuel, in munitions, in consumer products such as fireworks and highway flares, and in Chilean nitrate fertilizer which contains high natural concentrations of ClO_4^-. Evidence of ClO_4^- at significant levels in other fertilizers, some of which may contain Chilean nitrate, has been inconsistent (e.g. Susarla et al., 1999, 2000b; Urbansky et al., 2000b; Vogt and Jackson, 2010). The current ambient distribution of ClO_4^-

☆ An evaluation of the role of tropospheric ozone in phytoaccumulation of perchlorate reveals large interspecific differences in accumulation but no relationship with ozone exposure or absorbed ozone dose.
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does not correspond with the abundance of known point sources, suggesting that additional area sources may remain to be identified.

The role of tropospheric O_3 in the origin of non-anthropogenic ClO_4^- remains unclear. Tropospheric O_3 has increased in concentration since pre-industrial times (Vingarzan, 2004; Stevenson et al., 2006). Current levels of ambient O_3 are injurious to crop species and to native vegetation (Avnery et al., 2011; Booker et al., 2009; USEPA, 2013). High concentrations of O_3 have been shown experimentally to produce ClO_4^- from Cl^- in both aqueous solution and in dry systems (Dasgupta et al., 2005; Kang et al., 2006, 2008; Rao et al., 2010). Stable isotopic composition of some indigenous ClO_4^- in the US and Chile exhibits a significant $\Delta^{17}O$ anomaly, suggesting some production of natural ClO_4^- through O_3 mediated oxidation reactions. However, other sources appear to have a small O_3 mediated contribution (Bohlke et al., 2005; Jackson et al., 2010). Our preliminary evidence (Burkey et al., unpublished) shows that O_3-sensitive and O_3-tolerant genotypes of snap bean (*Phaseolus vulgaris*) accumulate foliar ClO_4^- under field conditions, and the role of contrasting O_3 environments is currently being evaluated. Through abscission and litter turnover this would represent an unaccounted source of ClO_4^- in the environment.

Plants, particularly in arid environments, may contain abundant chloride in their tissues; display a vast array of hydrated internal and external reaction surfaces; and catalyze a multitude of redox reactions that could be involved in biosynthesis of ClO_4^-. These factors, the ubiquitous distribution of plants, and the post-industrial increase in O_3 exposure are consistent with the possibility that tropospheric O_3 may induce biosynthesis of ClO_4^- from chloride in plants. This would represent a novel source of ClO_4^- in the environment.

We present the results of a series of experimental exposures to environmentally relevant concentrations of O_3 of a broad range of contrasting food, feed and fiber species under controlled conditions. We test the hypotheses that (1) exposure of plants to O_3 leads to foliar biosynthesis of ClO_4^- in young, physiologically active leaves, that (2) such exposure leads to accumulation of ClO_4^- in older, senescing leaves, and that (3) contrasting plant species exhibit little foliar ClO_4^- at low O_3 exposure.

2. Materials and methods

2.1. Plant material

We have chosen a range of plants of economic importance, used for human consumption, animal feed, or fiber. These species represent diverse classes of crop species, leafy green vegetable row crops and extensively cultivated grain and forage crops, warm season and cool season crops, and both C_3 and C_4 species.

The C_3 species were spinach (*Spinacia oleracea* cv. Bloomsdale Long Standing; Ferry Morse Seed Co., Fulton KY), lettuce (*Lactuca sativa* cv. Romaine, Parris Island Cos; Ferry Morse Seed Co., Fulton KY), broccoli (*Brassica oleracea* cv. De Cicco, Ferry Morse Seed Co., Fulton KY), soybean (*Glycine max* cv. Disoy; Ferry Morse Seed Co., Fulton KY), Pima cotton (*Gossypium barbadense* cv. Phytogen 800, Dow AgroScience, Indianapolis IN and cv. S-6, J.G. Boswell Company, Corcoran CA; foundation seed stock), and bush bean (*Phaseolus vulgaris* cv. Bush Blue Lake 156; Ferry Morse Seed Co., Fulton KY), The C_4 species were sorghum (*Sorghum bicolor* cv. 4662, Pioneer Seed Co., Johnston IA), sugarcane (*Saccharum oficinarum* × *S. spontaneum* hybrid cv. Elephant; Grantz and Vu, 2009; Grantz et al., 2012), and maize (*Zea mays* cv. Golden Cross Bantam (hybrid); Ferry Morse Seed Co., Fulton KY).

Seed (stalk cuttings in the case of sugarcane) were planted in moist commercial potting mix (Earthgro Potting Soil; Scotts Company, Marysville, OH) in 10 cm square pots. After emergence, pots were thinned to 1 plant per pot. Plants were grown in a research greenhouse at Kearney Research and Extension Center (103 msl; 36.598 N 119.503 W). Irrigation was provided daily through a drip emitter in each pot. A complete (N–P–K; 24-8-16) fertilizer solution was administered twice weekly (2.9 g L^{-1}, Miracle Gro, Scotts Miracle-Gro Products Inc., Port Washington, NY) through the same emitters. Both irrigation and fertilizer were applied until substantial drainage through the potting medium occurred, to avoid accumulation of salts or fertilizer in the soil (Grantz et al., 2010). Pots retained 68.9 mL of solution against drainage.

Plants were grown from germination until harvest in one of nine continuously stirred, Teflon lined tank reactors (CSTRs; Heck et al., 1978; Grantz et al., 2010) located in the greenhouse. Growth temperature was 15–30 °C, illuminated with natural sunlight (approximately 300 μmol m^{-2} s^{-1} PPFD; 400–700 nm at plant level) near solar noon.

2.2. Ozone exposure

Plants were exposed to environmentally relevant O_3 concentrations (12 h means nominally 4, 59, and 114 ppb; 8 h means of 4, 75 and 150 ppb, and daily maxima near solar noon of 4, 89 and 163 ppb) from emergence in the CSTRs. Exposures were imposed as daily half-sine wave, 7 days per week. O_3 was provided to the CSTRs by corona discharge (Model SGC-11, Pacific O_3 Technology, Brentwood, CA) from purified oxygen (Series ATF-15, Model 1242, SeQual Technologies Inc., San Diego CA). Feedback for the O_3 generator was provided by the exit stream of a single exposure chamber, monitored with an ultraviolet O_3 monitor (ThermoElectron Model 41C), with other CSTRs controlled by ratio of O_3 flow rate (Grantz et al., 2010). Each CSTR was monitored every 15 min, independently of the control system, with a separate ThermoElectron Model 41C. All monitors were calibrated against a factory certified calibration unit (Model 306; 2B Technologies; Boulder CO). Air with the desired O_3 concentration was introduced at one complete air exchange per minute.

2.3. Perchlorate determination

Plants were harvested at about 9 weeks after germination. Species varied with their specific rate of development, but all runs within a species were harvested at precisely the same plant age. Roots were washed in cool water to remove the potting medium. Leaves, roots and stems were separated and immediately frozen at −20 °C. Older leaves, senescing or recently abscised, were gathered separately and treated similarly.

Samples of unused planting media and fertilizer were collected and stored at −20 °C in zip-lock polyethylene bags. The surface 1 cm of soil was sampled following plant harvest and treated similarly. Irrigation water was sampled directly from the emitters of the drip irrigation system into plastic, screw-top vials and immediately frozen at −20 °C. Samples were shipped on dry ice to the analytical laboratory for ClO_4^- analysis.

Soil samples were extracted using Milli-Q water at a 2:1 mass ratio (water:soil) by shaking for 24 h. The samples were centrifuged for 10 min and the supernatant decanted and filtered through a 0.2 micron Nylon membrane (ion chromatography (IC)-certified Acrodisc syringe filter). All extraction sets were accompanied by an extraction duplicate, an extraction spike (soil + known amount of added ClO_4^-), and an extraction blank (DDI water only). The moisture content of parallel samples was determined by drying at 105 °C for 24 h. The final filtered extract was analyzed for major anions and ClO_4^-.

Plant leaf samples were pre-dried (105 °C for 12 h) and approximately 1 g placed in a 45-mL capacity centrifuge tube to which 25 mL of Milli-Q water was added. The centrifuge tubes, containing the samples, were boiled for 1 h (water bath temperature ~ 99 °C) and centrifuged at 5000 rpm for 5 min. A 2 mL aliquot of the supernatant was gently transferred into a plastic bottle containing 1.0 ± 0.1 g of activated alumina. The alumina-extract mixture was diluted by adding 18 mL of DDI water, capped, and held at 3 °C for 8 h. The suspension was then re-centrifuged at 5000 rpm for 5 min, and the final supernatant filtered (0.2 micron) and passed through a pre-cleaned and activated OnGuard® RP cartridge (Dionex Corporation). The extraction procedure was repeated for the extraction duplicate, spike and blank (DDI). The final solution was then diluted and analyzed for ClO_4^-.

Perchlorate in the resulting solutions was quantified by loading through a 25 μL pre-concentrating loop into an ion chromatograph-tandem mass spectrometer (IC-MS/MS with GP50 pump, CD25 conductivity detector, AS40 automated sampler and Dionex IonPac AS16 (250 × 2 mm) analytical column). This was coupled with an Applied Biosystems – MDS SCIEX API 2000® triple quadrupole mass spectrometer with a Turbo-IonSpray™ source. A 45 mM hydroxide (NaOH) eluent at 0.3 ml min^{-1} was followed by 90% acetonitrile (0.3 ml min^{-1}) as a post-column solvent. To overcome matrix effects all samples were spiked with an oxygen-isotope (^{18}O) labeled ClO_4^- internal standard. The method detection limit (MDL) for ClO_4^- was 0.01 μM.

ClO_4^- content of tissue, potting medium, and fertilizer is reported as (μg (kg dry wt)$^{-1}$). ClO_4^- content of irrigation water and fertilizer solution is reported as (μg L^{-1}).

2.4. Ozone flux

Stomatal conductance of young, healthy, fully expanded leaves (leaf 0 and leaf 2) was determined with a porometer (LI 1600; LiCor Inc., Lincoln NE USA or AP4; Delta T Devices, Cambridge UK). Measurements were determined on both classes of leaves at 2 h intervals throughout the day, and on 2 occasions at 14 day intervals. Values were averaged from these 4 leaves as an estimate of stomatal conductance over time, developmental age, and leaf position. Conductance was converted from water vapor to O_3 (Massman and Grantz, 1995) and multiplied by mean O_3 concentration over the surrounding 2 h period. The products were summed diurnally over daylight hours and over the lifespan (germination to harvest) of each species to yield cumulative flux, or dose.

2.5. Statistical analysis

CSTRs were arrayed in three blocks parallel to windows and cooling fans. Plants were randomly assigned to individual exposure chambers. One CSTR per block was exposed to each of the three O_3 concentrations, with the CSTR taken as the unit of replication in a Randomized Complete Block Design. Two runs were conducted with each species ($n = 6$) except for broccoli ($n = 9$), Pima Cotton ($n = 9$; as data for two closely related cultivars were combined), and spinach ($n = 3$). Neither blocks nor runs were significant, and data were pooled. Older leaves were not available at harvest for all species. Stomatal conductance and flux calculations were not available for all runs, but were available for all species (Fig. 3).

Values of ClO_4^- were normalized by basal values observed at 4 ppb O_3 for consideration of the relationship between accumulation and potential sensitivity of ClO_4^- contents to O_3 exposure (Fig. 2B). For comparison of responses of tissue ClO_4^- content to O_3 exposure vs. O_3 dose, ClO_4^- values were normalized within each species by the median value of ClO_4^- across all O_3 exposures.

Each species was analyzed independently for response to O_3 exposure. Basal ClO_4^- content of all species at low O_3 concentration was subjected to independent analysis by ANOVA with reduced degrees of freedom to evaluate differences in accumulation between species. Analyses were conducted using SAS v. 9.3 (SAS Institute Inc.; Cary NC, 2002). Means separation ($P < 0.05$) by Duncan's Multiple Range Test and standard errors of the means were performed with PROC GLM and PROC MEANS. To address a potential positive relationship between variances and means in some data sets, the data were analyzed in their native form by ANOVA, and again after transformation as the square root. Neither yielded significant differences and only the native analysis is presented. Linear regression analyses of relationships between perchlorate and ozone exposure and concentration were performed using PROC REG.

3. Results

3.1. Sources of perchlorate

The plants were irrigated with water containing very little ClO_4^- (Table 1). Similarly, the commercial potting mix exhibited relatively low ClO_4^- content. The commercial fertilizer applied to all species contained substantial amounts of ClO_4^- on a dry weight basis (Table 1), but only 3.6 μg ClO_4^- L^{-1} in the dilute irrigation solution. Over the approximate 9 weeks of plant growth, each pot received a total of approximately 4.4 μg ClO_4^- from the fertilizer that was applied twice weekly, and an additional 2.6 μg ClO_4^- in irrigation water applied daily. These provided the principal source for ClO_4^- accumulation from the growth medium (Table 1).

3.2. Perchlorate accumulation

Large interspecific differences were observed in foliar concentrations of ClO_4^- averaged across all O_3 exposures (Fig. 1; Table 2). These appear to reflect physiological differences in uptake or exclusion of ClO_4^- present in the growth medium. Spinach accumulated approximately 700 μg (kg dry wt)$^{-1}$, the highest observed, while sugarcane accumulated less than 100 μg kg^{-1}. Other species were intermediate, with young leaf contents lying generally between 150 and 250 μg kg^{-1}. On average, plants accumulated about 7% of the applied ClO_4^- over their lifespan, much of the remainder being lost to drainage.

The results were similar for older, senescing leaves (Table 3) although ClO_4^- was accumulated to much higher levels in these leaves. Young leaves of broccoli exhibited contents near 400 μg kg^{-1} (Table 2) whereas older leaves averaged slightly over 1000 μg kg^{-1} (Table 3). For sugarcane, the corresponding values were about

Table 1
ClO_4^- content of unused irrigation water, potting medium, and fertilizer.

Material	*n*	Perchlorate content	S.E.	Range
Irrigation water	11	0.61 (μg L^{-1})	±0.053	0.33–0.81
Potting medium	12	3.78 (μg (kg dry wt)$^{-1}$)	±0.72	0.43–6.41
Fertilizer (granular)	6	1270 (μg (kg dry wt)$^{-1}$)	±89.3	1090–1700
(as applied)		3.55 (μg L^{-1})	±0.25	

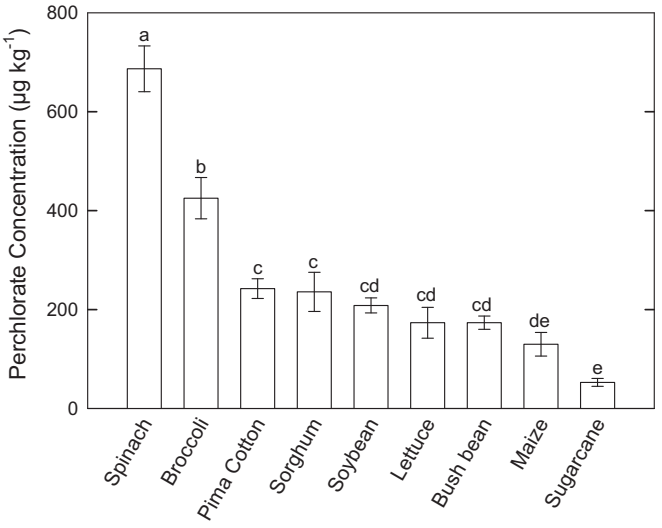

Fig. 1. Inter-specific differences in perchlorate content (μg (kg dry wt)$^{-1}$) of youngest fully expanded leaves of a range of crop species (mean over all O_3 exposures ± SE). Bars with different letters differ at $P < 0.05$.

50 μg kg^{-1} for young leaves (Table 2) but >100 μg kg^{-1} for older leaves (Table 3). Averaged over all species, older leaves accumulated more than twice the ClO_4^- as younger leaves (>500 μg kg^{-1} vs. <250 μg kg^{-1}).

Table 2
Perchlorate content (μg kg^{-1}) of young leaves of a range of crop species as a function of O_3 exposure (ppb, 12 h mean) sampled at the time of final harvest. There were no significant differences between O_3 exposures ($P < 0.05$) within any species, nor within the pooled data.

Species	*n*	O_3 exposure (ppb)	Perchlorate content (μg kg^{-1})	S.E.	Range
Bush bean	6	4	155	±31.3	72.1–294
	6	59	160	±19.0	110–225
	6	114	204	±15.4	130–236
Broccoli	9	4	380	±41.4	234–653
	7	59	349	±75.0	42.1–672
	9	114	528	±86.1	68.3–927
Lettuce	9	4	223	±89.0	65.8–926
	9	59	145	±26.3	56.2–279
	9	114	151	±20.1	81.3–250
Maize	6	4	173	±54.1	15.0–371
	5	59	85	±10.2	50.1–114
	6	114	123	±38.3	23.9–295
Pima cotton	18	4	290	±40.2	33.4–544
	18	59	218	±24.8	75.5–416
	18	114	218	±36.3	58.6–590
Sorghum	6	4	177	±38.4	87.5–328
	6	59	182	±20.6	128–267
	5	114	369	±110.3	84.2–598
Soybean	5	4	222	±39.8	136–366
	6	59	218	±16.8	164–284
	6	114	186	±24.7	123–294
Spinach	3	4	766	±84.5	670–934
	2	59	568	±110.6	458–679
	3	114	686	±31.8	622–719
Sugarcane	6	4	42.5	±6.2	30.6–71.4
	6	59	61.0	±19.0	6.67–132
	6	114	54.6	±15.3	15.0–122
All species	68	4	255	±24.8	15.00–934
	65	59	199	±17.0	6.67–679
	68	114	255	±26.1	15.0–927

Table 3
Perchlorate content of older, senescing leaves of a range of crop species as a function of O_3 exposure (ppb, 12 h mean) sampled at the time of final harvest. There were no significant differences between O_3 exposures ($P < 0.05$) within any species, nor within the pooled data.

Species	*n*	O_3 exposure (ppb)	Perchlorate content (μg kg^{-1})	S.E.	Range
Broccoli	9	4	1730	±970	312–9450
	8	59	765	±123	423–1430
	8	114	945	±170	417–1960
Lettuce	2	4	337	±188	150–525
	3	59	430	±171	165–750
	9	114	319	±62.5	188–750
Maize	6	4	87.0	±19.8	51.5–184
	6	59	85.1	±14.0	17.4–110
	5	114	77.4	±11.6	54.9–120
Pima cotton	12	4	435	±133	97.5–1320
	17	59	352	±74.6	78.1–1280
	18	114	549	±170	70.3–2330
Sorghum	3	4	238	±11.8	214–250
	3	59	527	±166.4	348–860
	3	114	463	±77.3	375–617
Sugarcane	3	4	131	±22.7	104–176
	3	59	85. 7	±16.0	58.4–114
	6	114	95.4	±7.94	72.1–125
All species	35	4	660	±267	51.5–9450
	40	59	394	±55.2	17.4–1432
	49	114	462	±78.7	54.9–2330

3.3. Effect of ozone exposure on perchlorate accumulation

There was no consistent effect of O_3 exposure on foliar content of ClO_4^- in young leaves. Five of nine species exhibited a decline in ClO_4^- content with increasing O_3 exposure while for the remaining four species ClO_4^- content increased with O_3 exposure. In no species was this response significant (Table 2) and even with the additional statistical power of combining all species ($n = 68$), the mean response across all species was not significantly related to O_3 exposure (Table 2). The same absence of relationship with O_3 exposure was observed in older leaves, despite the greater overall accumulation of ClO_4^- (Table 3).

Basal accumulation of ClO_4^- at low O_3 was not predictive of the effect of elevated O_3, on ClO_4^- in young leaves, whether significant or not (Fig. 2; Table 4). This was the case for absolute changes in ClO_4^- between low and moderate O_3 and between low and higher O_3 exposures (Fig. 2A; triangles, squares, respectively). The relationship was not improved by normalization of the values associated with O_3 exposure by basal ClO_4^- content (Fig. 2B).

We considered whether O_3-induced foliar biosynthesis of ClO_4^- might occur, yet not be reflected in leaf contents due to transport to the rhizosphere. However, there was no relationship between ClO_4^- in the top level of the potting medium and basal accumulation of ClO_4^- at 4 ppb O_3 (Table 4). Similarly, there was no significant relationship between O_3 exposure of plant and potting medium and surface content of ClO_4^- (Table 4). However, a multiple range test of ClO_4^- in the surface layer of the growth medium of unused potting medium, potting medium exposed at 4 ppb O_3, and potting medium exposed at 114 ppb O_3 (Table 5) suggested a positive association between ClO_4^- and O_3. The potting medium exposed to the highest and lowest O_3 concentrations did not differ in ClO_4^- content, and that exposed to the lowest O_3 did not differ from the unused medium. However, there was a significant difference between unused potting medium and that exposed to the highest O_3 concentration.

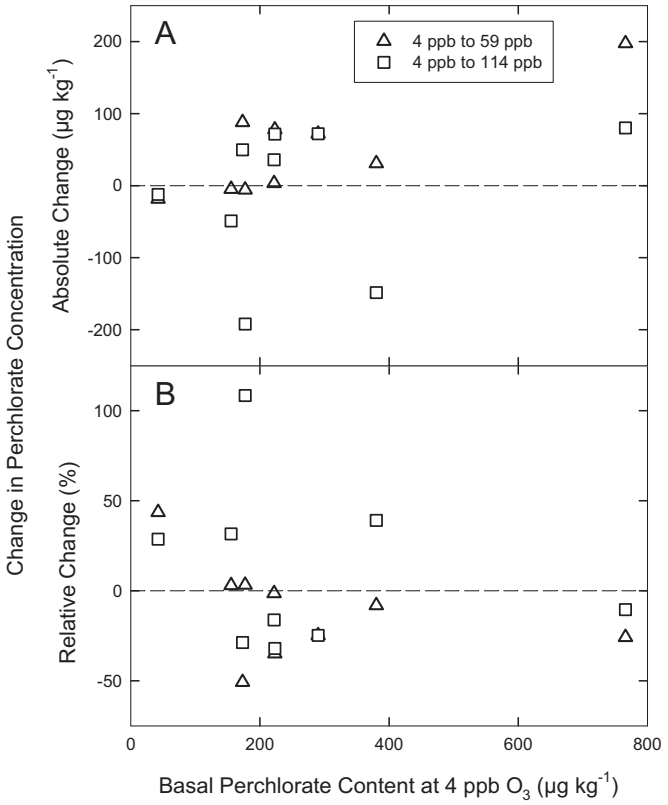

Fig. 2. There was no significant relationship between basal perchlorate content (at 4 ppb O_3) and the change in ClO_4^- content (unitless) between 4 and 59 ppb O_3 (circles) and between 4 and 114 ppb (squares) of youngest fully expanded leaves of a range of crop species.

3.4. Role of ozone metrics

We evaluated whether the lack of response of foliar ClO_4^- content in young and older leaves to O_3 exposure was associated with a failure of the exposure protocol. This was not supported by the observation that these plants exhibited a highly significant reduction in above ground biomass at the highest O_3 exposure (data not shown).

We also considered whether the use of O_3 concentration could be an inadequate metric of O_3 exposure. The lack of relationship between foliar ClO_4^- and O_3 was not improved by use of cumulative stomatal uptake of O_3 (O_3 dose) rather than O_3 concentration (O_3 exposure). A linear regression analysis of the subset of young leaf data for which stomatal conductance was available (Fig. 3) revealed a non-significant relationship between normalized ClO_4^- and O_3 exposure (Fig. 3A), consistent with the results of the ANOVA with young leaves (Table 2). The relationship was not improved when ClO_4^- was considered as a function of O_3 dose (Fig. 3B).

4. Discussion

4.1. Perchlorate accumulation

The plants in the current study exhibited a range of accumulation factors for ClO_4^- in the growth medium. This is consistent with previous studies showing that plants accumulate ClO_4^- with bioconcentration factors of up to two orders of magnitude (Tan et al., 2004, 2005; Urbansky et al., 2000; van Aken and Schnoor, 2002).

Uptake of ClO_4^- has been observed in many species, including in cottonwood (*Populus deltoids*, hybrid *Populus*), *Eucalyptus cineria*,

Table 4
Perchlorate content (μg (kg dry wt)$^{-1}$) of potting medium after use for plant growth by a range of crop species, sampled at the time of final harvest (mean ± S.E.), as a function of O_3 exposure (ppb, 12 h mean). There were no significant differences between O_3 exposures ($P < 0.05$) within any species, nor within the pooled data.

Species	*n*	O_3 exposure (ppb)	Perchlorate content (μg kg^{-1})	S.E.	Range
Broccoli	6	4	5.68	±0.40	5.00–7.61
	6	59	6.09	±0.26	5.38–7.07
	6	114	6.2	±0.39	4.62–7.31
Bush bean	3	4	6.98	±0.08	6.88–7.14
	3	59	6.53	±0.2	6.15–6.82
	3	114	6.14	±0.25	5.88–6.64
Lettuce	6	4	6.13	±0.27	5.49–7.34
	6	59	5.7	±0.21	5.07–6.36
	6	114	7.32	±1.08	6.00–12.63
Maize	3	4	0.51	±0.05	0.43–0.60
	1	59	2.8	na	2.80–2.80
	3	114	0.44	±0.06	0.37–0.55
Pima cotton	9	4	1.38	0.29	0.65–2.87
	8	59	3.44	2.02	0.69–17.5
	9	114	4.49	2.03	1.03–18.6
Sorghum	3	4	7.79	±0.88	6.70–9.52
	3	59	7.16	±0.09	6.98–7.25
	3	114	8.65	±1.32	7.32–11.3
Soybean	3	4	6.96	±0.31	6.47–7.54
	3	59	6.65	±0.61	5.54–7.65
	3	114	6.41	±0.25	5.95–6.82
Spinach	3	4	5.73	±0.21	5.34–6.07
	3	59	6.05	±0.87	4.59–7.60
	3	114	6.26	±0.17	5.93–6.49
Sugarcane	3	4	10.7	±3.18	4.53–15.1
	3	59	7.55	±0.43	6.81–8.31
	3	114	9.31	±1.83	5.70–11.6
All species	39	4	5.11	±0.53	0.43–15.1
	36	59	5.63	±0.50	0.69–17.5
	39	114	5.98	±0.61	0.37–18.6

willow (*Salix nigra*) (Nzengung et al., 1999) and in tamarisk (*Tamarix ramosissima*) (Urbansky et al., 2000a), cucumber (*Cucumis sativus*), lettuce, and soybean (Yu et al., 2004; Yang and Her, 2011), and many others. The high tissue contents observed in spinach (about 700 μg kg^{-1}) are consistent with previous reports for leafy green food crops.

In our study this ClO_4^- was contributed mostly by the commercial fertilizer. Although ClO_4^- from this material did not accumulate significantly in the potting medium, it was apparently available during and following the twice weekly application. Under field conditions diverse plant species demonstrated a substantial capacity for phyto-accumulation of ClO_4^- (Tan et al., 2004; Yu et al., 2004; Jackson et al., 2005), with the magnitude of uptake related to the distance from streams draining ClO_4^- contaminated watersheds and to the duration of exposure (Tan et al., 2004, 2005). This capacity for uptake suggests that phytoremediation of contaminated

Table 5
Perchlorate content (μg (kg dry wt)$^{-1}$) of potting medium before and after use for plant growth, sampled directly from the commercial container or from pots of all species at the time of final harvest (mean ± S.E.), as a function of O_3 exposure (ppb, 12 h mean). Means followed by different letters were significantly different ($P < 0.05$).

Exposure (ppb)	*n*	Perchlorate content (μg kg^{-1})	S.E.	Range (μg kg^{-1})
na	12	3.78a	±0.72	0.43–6.41
4	39	5.11ab	±0.53	0.43–15.1
114	39	5.98b	±0.61	0.37–18.6

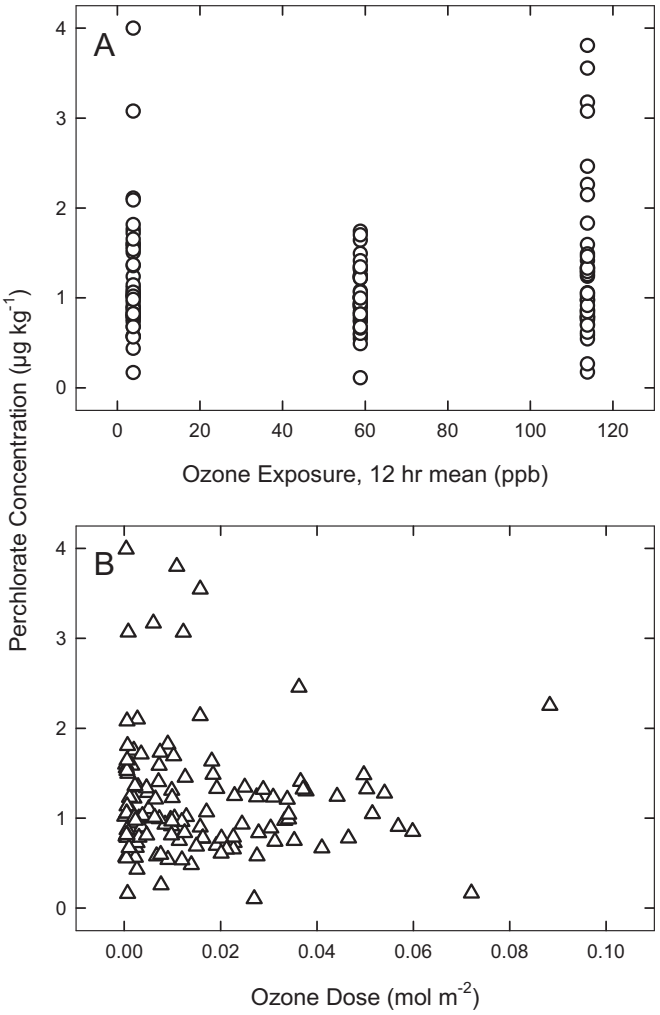

Fig. 3. There was no significant relationship between O_3 exposure (A; $r^2 = 0.0034$) or O_3 dose (cumulative flux; B; $r^2 = 0.0018$) and perchlorate content of young leaves normalized by the median perchlorate content of each species shown in Fig. 1 for which O_3 flux data were available.

watersheds may be feasible. Our data and these previous studies indicate that this phyto-accumulation, rather than biosynthesis, appears to account for the appearance of ClO_4^- in the human food supply.

The contrasting accumulation characteristics among species in this and previous studies appear to reflect physiological differences in uptake or exclusion by roots of ClO_4^- present in the growth medium. Phytoaccumulation of ClO_4^- occurs in transpiring leaves, apparently due to transport in the xylem transpiration stream (Seyfferth et al., 2007). Accumulation in leaf tissue has been effectively modeled using growth and passive (i.e. first order) uptake kinetics (Seyfferth et al., 2008a; Sundberg et al., 2003). In the present and previous studies, root and stem tissue of all species exhibited very low ClO_4^- contents relative to leaves (Vogt and Jackson, 2010). Bioaccumulation was considerably higher in leaves than in pods or fruits of soybean and tomato (Jackson et al., 2005).

In the juvenile plants used in the present study it was relatively simple to distinguish young, healthy, fully expanded leaves from the older and senescing cohort. Under these conditions we demonstrate that the older leaf population also accumulated ClO_4^-, and to a considerably greater extent than the younger leaves. It is not known if this reflects the greater age of the leaf for

accumulation by transpiration, or a physiological sequestering of this xenobiotic in older leaves soon to be shed from the plant body. In any case, over all species the older leaves accumulated more than twice as much ClO_4^- as younger leaves. This represents a potent mechanism for concentrating ClO_4^- from the rhizosphere to the soil surface.

4.2. Effect of ozone exposure on perchlorate accumulation

O_3 exposure had a nearly random effect on foliar content of ClO_4^- in both young and older leaves. Approximately half of experimental species exhibited a decline in ClO_4^- content with increasing O_3 exposure, while the other half exhibited an increase. These trends were not significant in any individual species nor in the combined, all-species, data set.

Our attempts to improve the power of the test of O_3 effects were not successful. Basal accumulation of ClO_4^-, indicative of favorable uptake/unfavorable exclusion properties of root membranes, was not predictive of the effect of O_3 on ClO_4^- content of young leaves. This putative relationship was tested at moderate and at higher O_3 exposures without success. Similarly, various normalization procedures, seeking to remove the undue influence of high baseline values of ClO_4^- in the accumulating species did not improve relationships between O_3 exposure and changes in tissue ClO_4^-.

The O_3 exposure protocol and its representation as O_3 concentration was adequate to induce a substantial decline in above ground biomass in these plants, suggesting that the test could have identified O_3-induced biosynthesis of ClO_4^- if it had occurred. The uptake of ClO_4^- observed in this study is not without potential consequence for plants. Millimolar concentrations of ClO_4^- in irrigation water reduced photosynthetic electron transport and induced antioxidant metabolism in tobacco and *Arabidopsis* (Hamissou, 2011). As ClO_4^- is not significantly metabolized in plants (Seyfferth et al., 2008b; Susarla et al., 2000a) it is unclear whether this induction of ascorbate peroxidase and superoxide dismutase reflects the strongly oxidizing nature of ClO_4^-, or a non-specific toxic effect of this xenobiotic. In either case, we can conclude from the present data that the growth inhibition associated with O_3 exposure is not a consequence of enhanced tissue accumulation of ClO_4^-, but rather reflects the phytotoxicity of O_3, itself. The data were clear in indicating that ambient and near ambient concentrations of O_3 did not lead to increased tissue contents of ClO_4^-.

4.3. Alternative sinks

We observed bioconcentration of ClO_4^- in older, senescing and abscised leaves. In more mature canopy conditions this would serve to shed ClO_4^- from the plant and distribute it onto the soil surface. As we collected these leaves either prior to abscission or soon afterwards and prior to decomposition or leaching, this mechanism did not apply in the present study.

The soil surface and any Cl^- in the irrigation water or fertilizer were exposed directly to O_3 in the CSTRs. There was some indication that direct exposure of the growth medium to the highest O_3 concentration may have led to accumulation of ClO_4^- in the surface layer. Averaged over all species there was a modest but non-significant relationship with increasing O_3 exposure, but a significant difference between unused potting medium and medium exposed to the highest O_3. This suggests that ClO_4^- content in the soil surface may increase with exposure to O_3, presumably due to oxidation of Cl^- in the soil, as demonstrated experimentally at higher O_3 concentrations with Cl^- coated sand (Kang et al., 2008) and soil (Dasgupta et al., 2005). Our results were observed without artificial enhancement of the Cl^- content, at near ambient O_3 concentrations, and over relatively brief exposure periods relative to geologic time. This potentially important conclusion requires confirmation, but if reproducible and applicable under field conditions, this mechanism would contribute to ClO_4^- present in the environment.

The clear absence of O_3-sensitivity of ClO_4^- in young leaves could have indicated a robust translocation to roots and exudation into the rhizosphere. However, we observed no relationship between foliar accumulation of ClO_4^- and its content in the potting medium. Retranslocation to stems or roots has not been detected in previous studies (Vogt and Jackson, 2010), consistent with high concentrations of ClO_4^- in leaf laminae and considerably lower concentrations in the rest of the plant. This was the case in the current study, and in *Polygonum* spp. (smartweed) in which leaves accumulated up to 800 μg (kg dwt)$^{-1}$, while roots and stems accumulated only 100–150 μg (kg dwt)$^{-1}$ (Tan et al., 2006). Neither exudation to non-contaminated media nor reductive metabolism are significant sinks for phytoaccumulated ClO_4^-, (van Aken and Schnoor, 2002), though some metabolites were detected in *Populus*.

5. Conclusion

The ubiquitous distribution of vegetation and rising concentrations of tropospheric O_3 provided a tempting hypothesis to explain the quantitatively and spatially inadequate emission inventory for ClO_4^- in the environment. We show that a broad range of crop species accumulate ClO_4^- from the growth medium, differing widely in their effectiveness in bioconcentration. Foliar ClO_4^- concentration was greatest in older leaves, which ultimately contribute to the litter layer, suggesting that scavenging of ClO_4^- from deeper soil horizons could lead to redistribution on the soil surface. However, we found no evidence that exposure of leaves to ambient O_3 induces any increase in tissue contents of ClO_4^-. We found an increasing trend in soil surface ClO_4^- with increasing O_3, and a significant difference between potting medium exposed to high O_3 and unexposed medium. The environmental significance of this result is not known. These results demonstrate that current ambient concentrations of O_3 in most locations do not lead to increased phyto-accumulation nor biosynthesis of ClO_4^-. They do not disprove the hypothesis that such plant activity could be induced by the higher O_3 concentrations observed in some areas of the developing world and during stratospheric incursions, or in potential future atmospheres.

References

Avnery, S., Mauzerall, D.L., Liu, J., Horowitz, L.W., 2011. Global crop yield reductions due to surface ozone exposure: 1. Year 2000 crop production losses and economic damage. Atmospheric Environment 45, 2284–2296.

Böhlke, J.K., Sturchio, N.C., Gu, B., Horita, J., Brown, G.M., Jackson, W.A., Batista, J.R., Hatzinger, P.B., 2005. Perchlorate isotope forensics. Analytical Chemistry 77, 7838–7842.

Booker, F., Muntifering, R., McGrath, M., Burkey, K., Decoteau, D., Fiscus, E., Manning, W., Krupa, S., Chappelka, A., Grantz, D., 2009. The ozone component of global change: potential effects on agricultural and horticultural plant yield, product quality and interactions with invasive species. Journal of Integrative Plant Biology 51, 337–351.

Dasgupta, P.,K., Martinelango, P.K., Jackson, W.A., Anderson, T.A., Tian, K., Tock, R.W., Rajagopalan, S., 2005. The origin of naturally occurring perchlorate: the role of atmospheric processes. Environmental Science and Technology 39, 1569–1575.

Dasgupta, P.,K., Dyke, J.V., Kirk, A.B., Jackson, W.A., 2006. Perchlorate in the United States. Analysis of relative source contributions to the food chain. Environmental Science and Technology 40, 6608–6614.

Grantz, D.A., Vu, H.-B., 2009. O_3 Sensitivity in a potential C_4 bioenergy crop: sugarcane in California. Crop Science 49, 643–650.

Grantz, D.A., Vu, H.-B., Aguilar, C., Rea, M.A., 2010. No interaction between methyl jasmonate and ozone in pima cotton: growth and allocation respond independently to both. Plant Cell and Environment 33, 717–728.

Grantz, D.A., Vu, H.-B., Tew, T.L., Veremis, J.C., 2012. Sensitivity of gas exchange parameters to o_3 in diverse C_4 sugarcane hybrids. Crop Science 52, 1–11.

Hamissou, M., 2011. Selected physiological and molecular responses of *Arabidopsis thaliana* and *Nicotiana tobacum* plants irrigated with perchlorate-containing water. Asian Journal of Plant Sciences 10, 255–262.

Heck, W.W., Philbeck, R.B., Denning, J.A., 1978. A Continuous Stirred Tank Reactor (CSTR) System for Exposing Plants to Gaseous Air Pollutants. USDA, Washington DC. Pub. No. ARS-563 5-181.

Jackson, W.A., Joseph, P.C., Patil, L.B., Tan, K., Smith, P.N., Yu, L., Anderson, T.A., 2005. Perchlorate accumulation in forage and edible vegetation. Journal of Agricultural and Food Chemistry 53, 369–373.

Jackson, W.A., Böhlke, J.K., Gu, B., Hatzinger, P.B., Sturchio, N.C., 2010. Isotopic composition and origin of indigenous natural perchlorate and co-occurring nitrate in the southwestern United States. Environmental Science and Technology 44, 4869–4876.

Jaegle, L., Yung, Y.L., Toon, G.C., Sen, B., Blavier, J.F., 1996. Balloon observations of organic and inorganic chlorine in the stratosphere: the role of $HClO_4$ production on sulfate aerosols. Geophysical Research Letters 23, 1749–1752.

Kang, N., Anderson, W., Jackson, W.A., 2006. Photochemical formation of perchlorate from aqueous oxychlorine anions. Analytica Chimica Acta 567, 48–56.

Kang, N., Jackson, W.A., Dasgupta, P.K., Anderson, T.A., 2008. Perchlorate production by ozone oxidation of chloride in aqueous and dry systems. Science of the Total Environment 405, 301–309.

Massman, W.J., Grantz, D.A., 1995. Estimating canopy conductance to ozone uptake from observations of evapotranspiration at the canopy scale and at the leaf scale. Global Change Biology 1, 183–198.

Michalski, G., Bohlkem, J.,K., Thiemens, M., 2004. Long term atmospheric deposition as the source of nitrate and other salts in the Atacama Desert, Chile: new evidence from mass-independent oxygen isotopic compositions. Geochimica Cosmochimica Acta 68, 4023–4038.

Nzengung, V.A., Wang, C., Harvey, B., 1999. Plant-mediated transformation of perchlorate into chloride. Environmental Science and Technology 33,1470–1478.

Rajagopalan, S., Anderson, T.A., Fahlquist, L., Rainwater, K.A., Ridley, M., Jackson, A.W., 2006. Widespread presence of naturally occurring perchlorate in High Plains of Texas and New Mexico. Environmental Science and Technology 40, 3156–3162.

Rao, B., Anderson, T.A., Orris, G.J., Rainwater, K.A., Rajagopalan, S., Sandvig, R.M., Scanlon, B.R., Stonestrom, D.A., Walvoord, M.A., Jackson, W.A., 2007. Widespread natural perchlorate in unsaturated zones of the southwest United States 41, 4522–4528.

Rao, B., Anderson, T.A., Redder, A., Jackson, W.A., 2010. Perchlorate formation by ozone oxidation of aqueous chlorine/oxy-chlorine species: role of Cl_xO_y radicals. Environmental Science and Technology 44, 2961–2967.

Rao, B., Mohan, S., Neuber, A., Jackson, W.A., 2012. Production of Perchlorate by Laboratory Simulated Lightning Process 223, 275–287.

Seyfferth, A.L., Henderson, M.K., Parker, D.R., 2008a. Effects of common soil anions and pH on the uptake and accumulation of perchlorate in lettuce. Plant and Soil 302, 139–148.

Seyfferth, A.L., Sturchio, N.C., Parker, D.R., 2008b. Is perchlorate metabolized or retranslocated within lettuce leaves? Environmental Science and Technology 42, 9437–9442.

Seyfferth, A.L., Parker, D.R., 2007. Effects of genotype and transpiration rate on the uptake and accumulation of perchlorate (ClO_4^-) in lettuce. Environmental Science and Technology 41, 3361–3367.

Stevenson, D.S., Dentener, F.J., Schultz, M.G., Ellingsen, K., van Noije, T.P.C., Wild, O., Zeng, G., Amann, M., Atherton, C.S., Bell, N., Bergmann, D.J., Bey, I., Butler, T., Cofala, J., Collins, W.J., Derwent, R.G., Doherty, R.M., Drevet, J., Eskes, H.J., Fiore, A.M., Gauss, M., Hauglustaine, D.A., Horowitz, L.W., Isaksen, I.S.A., Krol, M.C., Lamarque, J.-F., Lawrence, M.G., Montanaro, V., Muller, J.-F., Pitari, G., Prather, M.J., Pyle, J.A., Rast, S., Rodriguez, J.M., Sanderson, M.G., Savage, N.H., Shindell, D.T., Strahan, S.E., Sudo, K., Szopa, S., 2006. Multimodel ensemble simulations of present-day and near-future tropospheric ozone. Journal of Geophysical Research 111, D08301. http://dx.doi.org/10.1029/2005JD006338.

Sundberg, S.E., Ellington, J.J., Evans, J.J., Keys, D.A., Fisher, J.W., 2003. Accumulation of perchlorate in tobacco plants: development of a plant kinetic model. The Journal of Environmental Monitoring 5, 505–512.

Susarla, S., Bacchus, S.T., Harvey, G., McCutcheon, S.C., 2000a. Uptake and transformation of perchlorate by vascular plants. Toxicological and Environmental Chemistry 74, 29–47.

Susarla, S., Collette, T.W., Garrison, A.W., Wolfe, N.L., McCutcheon, S.C., 1999. Perchlorate identification in fertilizers. Environmental Science and Technology 33, 3469–3472.

Susarla, S., Collette, T.W., Garrison, A.W., Wolfe, N.L., McCutcheon, S.C., 2000b. Additions and corrections: "Perchlorate identification in fertilizers". Environmental Science and Technology 34, 224.

Tan, K., Anderson, T.A., Jackson, W.A., 2006. Uptake and exudation behavior of perchlorate in smartweed. International Journal of Phytoremediation 8, 13–24.

Tan, K., Anderson, T.A., Jackson, W.A., 2005. A temporal and spatial variation of perchlorate in streambed sediments: results from in-situ dialysis samplers. Environmental Pollution 136, 283–291.

Tan, K., Anderson, T.A., Jones, M.W., Smith, P.N., Jackson, W.A., 2004. Accumulation of perchlorate in aquatic and terrestrial plants at a field scale. Journal of Environmental Quality 33, 1638–1646.

Urbansky, E.T., Brown, S.K., 2003. Perchlorate retention and mobility in soils. Journal of Environmental Monitoring 5, 455–462.

Urbansky, E.T., Magnuson, M.L., Kelty, C.A., Brown, S.K., 2000a. Perchlorate uptake by salt cedar (*Tamarix ramosissima*) in the Las Vegas Wash riparian ecosystem. Science of the Total Environment 256, 227–232.

Urbansky, E.T., Magnuson, M.L., Kelty, C.A., Gu, B., Brown, G.M., 2000b. Comment on: "Perchlorate identification in fertilizers, and the subsequent addition/correction". Environmental Science and Technology 34, 4452–4453.

USEPA, February 2013. Integrated Science Assessment for Ozone and Related Photochemical Oxidants. EPA 600/R-10/076F. United States Environmental Protection Agency, Research Triangle Park, NC.

van Aken, B., Schnoor, J.L., 2002. Evidence of perchlorate (ClO_4^-) reduction in plant tissues (poplar tree) using radio-labeled (ClO_4^-)-^{36}Cl. Environmental Science and Technology 36, 2783–2788.

Vingarzan, R., 2004. A review of surface ozone background levels and trends. Atmospheric Environment 38, 3431–3442.

Vogt, W., Jackson, W.A., 2010. Perchlorate, nitrate, and iodine uptake and distribution in lettuce (Lactuca sativa L.) and potential impact on background levels in humans. Journal of Agricultural and Food Chemistry 58, 12192–12198.

Yang, M., Her, N., 2011. Perchlorate in soybean sprouts (*Glycine max* L. Merr.), water dropwort (*Oenanthe stolonifera* DC.), and Lotus (*Nelumbo nucifera* Gaertn.) root in South Korea. Journal of Agricultural and Food Chemistry 59, 7490–7495.

Yu, L., Canas, J.E., Cobb, G.P., Jackson, W.A., Anderson, T.A., 2004. Uptake of perchlorate in terrestrial plants. Ecotoxicology and Environmental Safety 58, 44–49.