Phase II: Identification and Characterization of Natural Sources of Perchlorate

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# Identification and Characterization of Natural Sources of perchlorate

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**ABSTRACT**
The objective of this research effort was to develop an improved understanding of (1) the distribution and isotopic characteristics of natural perchlorate worldwide, (2) the mechanisms of natural perchlorate production and (3) the contributing processes resulting in the ubiquitous distribution of this anion and its stable isotope characteristics in soils, groundwater, and vegetation. Data from the project reveal that natural perchlorate is widely distributed in soils and groundwater in arid and semi-arid environments worldwide. Natural perchlorate was also found to be the dominant source on this anion in the U.S. Great Lakes, at concentrations ranging from 0.05 to 0.13 μg/L. Both UV-photolysis and ozone mediated mechanisms may contribute to the formation of natural perchlorate and to its isotopic characteristics. Biological synthesis of perchlorate in bacteria or plants was not observed. However, many plant species were observed to bioaccumulate perchlorate, particularly in leaf tissue. The isotopic signature of this plant-accumulated perchlorate represented that of the dominant environmental source, potentially providing a means to identify sources in produce.

**SUBJECT TERMS:**
perchlorate, nitrate, chloride, UV-photolysis, ozone, stable isotope, oxygen-18, oxygen-17, chlorine-37, chlorine-36, plants, and, Great Lakes, ground water, synthesis, Atacama, Antarctica, southwest, bioaccumulation, CSLA, produce

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ACRONYM LIST

Acronyms and Abbreviations

‰ per mil
δ, Δ delta, relative difference of isotope ratios
ACS American Chemical Society
ANOV A Analysis of Variance
Ag silver
AgCl silver chloride
AgNO3 silver nitrate
Al aluminum
Al2O3 activated alumina
AMS accelerator mass spectrometry
Aq aqueous
Ar argon
Br- bromide
BV bed volumes
°C degrees Celsius
Ca calcium
ccSTP cubic centimeters at Standard Temperature and Pressure
CCL Contaminant Candidate List of USEPA
CFC chlorofluorocarbon
CF-IRMS continuous-flow isotope ratio mass spectrometry
CH4 methane
CH3Cl methyl chloride
CH3I methyl iodide
Cl- chloride
Cl chlorine
35Cl chlorine-35
36Cl chlorine-36
37Cl chlorine-37
ClO2 chlorine dioxide
ClO2- chlorite
ClO3- chlorate
ClO4- perchlorate
ClOx oxychlorine species
cm centimeters
CO carbon monoxide
CO2 carbon dioxide
COC chain of custody
CsCl cesium chloride
CsClO4 cesium perchlorate
CsOH cesium hydroxide
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KNO₃ potassium nitrate
KOH potassium hydroxide
L liter
LUB Lower Umatilla Basin
LC-MS/MS liquid chromatography-tandem mass spectrometry
µg microgram
µg/L microgram per liter
µm micron
µmol micromole
M molar
m meter
MDL method detection limit
MDV McMurdo Dry Valleys, Antarctica
Mg magnesium
mg milligram
MRGB Middle Rio Grande Basin
min minute
mL milliliter
mm millimeter
mM millimolar
MS mass spectrometry
Msl mean sea level
n amount of substance
N nitrogen or normal or number of entities
N₂ nitrogen gas
Na sodium
NaCl sodium chloride
NaClO₄ sodium perchlorate
NaOCl sodium hypochlorite (bleach)
NaOH sodium hydroxide
NASA National Aeronautics and Space Administration
Na₂S₂O₃ sodium thiosulfate
Ne neon
ng nanogram
NH₄ClO₄ ammonium perchlorate
NH₄ ammonia
Ni nickel
nL nanoliter
N₂O nitrous oxide
NO₂⁻ nitrite
NO₃⁻ nitrate
NO₃-N nitrate as nitrogen
NRC National Research Council of the National Academies of Science
O oxygen
$^{16}$O  oxygen-16
$^{17}$O  oxygen-17
$^{18}$O  oxygen-18
O$_2$  oxygen gas
O$_3$  ozone
OCl$^-$  hypochlorite

ORNL  Oak Ridge National Laboratory
ORP  oxidation-reduction potential
per mil (‰)  part per thousand ($\times 10^{-3}$)
ppb  part per billion ($\times 10^{-9}$)
pmc  percent modern carbon
ppm  part per million ($\times 10^{-6}$)
PO$_4^{3-}$  phosphate
PRIME  Purdue Rare Isotope Measurement Laboratory
QA/QC  Quality Assurance/Quality Control
RfD  reference dose
S  sulfur

SERDP  Strategic Environmental Research and Development Program
SHP  Southern High Plains
SMOC  Standard Mean Ocean Chloride
SO$_2$  sulfur dioxide
SO$_4^{2-}$  sulfate
TDS  total dissolved solids
TOC  total organic carbon
TU  tritium unit
µg  micrograms

UIC  University of Illinois at Chicago
U.S.  United States
UV  ultraviolet
USEPA  United States Environmental Protection Agency
USGS  U.S. Geological Survey
VOCs  volatile organic compounds
VSMOW  Vienna Standard Mean Ocean Water
WL  West Lobe Lake Bonney, Antarctica
x  mole fraction
yr  year
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EXECUTIVE SUMMARY

Objectives
Perchlorate (ClO$_4^-$) has both synthetic and natural sources. Stable isotope analysis of O ($\delta^{18}$O, $\delta^{17}$O) and stable ($\delta^{37}$Cl) and radioactive ($^{36}$Cl) isotope analysis of Cl in ClO$_4^-$ allows natural and synthetic ClO$_4^-$ to be distinguished from each other and provides information on the potential origin(s) of natural ClO$_4^-$. The goal of this research effort was to develop an improved understanding of (1) the distribution and isotopic characteristics of natural ClO$_4^-$ worldwide, (2) the mechanisms of natural ClO$_4^-$ production and (3) the contributing processes resulting in the ubiquitous distribution of this anion and its stable isotope characteristics in soils, groundwater, and vegetation.

Technical Approach
In order to achieve these goals, several different laboratory and field research tasks were undertaken. These objectives and tasks were designed to fill existing data gaps on the production, distribution and isotopic characteristics of natural ClO$_4^-$. The first tasks were designed to improve our understanding of ClO$_4^-$ distribution, significant environmental ClO$_4^-$ production mechanisms and the stable isotopic signature of the produced ClO$_4^-$. These tasks included the following: (1) determination of the isotopic signature of ClO$_4^-$ produced by O$_3$ and UV oxidation of Cl$^-$ and relevant ClO$_x$ species; (2) development of an improved understanding of the occurrence and isotopic signature of natural ClO$_4^-$ and its relationship with NO$_3^-$ and other anions in arid and semi-arid environments worldwide; and (3) evaluation of the potential for microbial production of ClO$_4^-$. A second set of tasks were designed to better understand plant accumulation of environmental ClO$_4^-$, whether its isotopic signature is altered during uptake and accumulation, and/or whether plants can actually generate ClO$_4^-$ via an O$_3$-mediated mechanism.

Results
The results from a range of different laboratory studies evaluating ClO$_4^-$ formation mechanisms confirm that there are multiple potential pathways of ClO$_4^-$ generation from both UV-photolysis and O$_3$-mediated oxidation of Cl$^-$ and other ClO$_x$ precursors. Laboratory studies were successful in producing Cl and O isotopic variations in ClO$_4^-$ that incorporate much of the reported stable isotope variation in natural ClO$_4^-$. Only the characteristic low $\delta^{37}$Cl values of Atacama ClO$_4^-$ (Chile) were not reproduced and these remain enigmatic. Data indicate that final ClO$_4^-$ isotopic composition is dependent on the precursor species oxidized. The reaction rates and intermediate species proposed to be involved in ClO$_4^-$ formation require further study and additional experiments are required to resolve the reason for the low $\delta^{37}$Cl values of Atacama ClO$_4^-$, but significant progress was made in constraining pathways of ClO$_4^-$ production in nature by application of stable isotope analysis.

Soil and groundwater sampling was conducted worldwide (including the continental US, South Africa, South America, China, Antarctica, and the Middle East) to evaluate concentrations of
ClO$_4^-$ and its relationship with common co-occurring anions such as Cl$^-$ and NO$_3^-$ among others. The data indicate that ClO$_4^-$ is globally distributed in soil and groundwater in arid and semi-arid regions on Earth at concentrations ranging from $10^1$ to $10^6$ µg/kg. Generally, the ClO$_4^-$ concentration in these regions increases with aridity index, but this also depends on the duration of arid conditions. In many arid and semi-arid areas, NO$_3^-$ and ClO$_4^-$ co-occur at consistent ratios (NO$_3^-/ClO_4^-$) that vary between $\sim 10^4$ and $\sim 10^5$. This is not the case for Cl$^-$/ClO$_4^-$ ratios, which vary widely among locations. The NO$_3^-/ClO_4^-$ ratios are largely preserved in hyper-arid areas that support little or no biological activity (e.g. plants or bacteria), but can be altered in areas with more active biological processes. In general, the co-occurrence of ClO$_4^-$ and NO$_3^-$ in arid and semi-arid locations, and associated variations in the isotopic composition of the NO$_3^-$, are consistent with a conceptual model of atmospheric origin, global co-deposition, and variable alteration of the NO$_3^-$ pool by biogenic addition, assimilation, and/or recycling on the surface. The Atacama Desert appears to be unique compared to other arid and semi-arid locations. There, exceptional enrichment in ClO$_4^-$ compared to Cl$^-$ or NO$_3^-$, accompanied by unique ClO$_4^-$ isotopic characteristics, may reflect an unusually efficient, but yet unknown, in situ production mechanism, regionally elevated atmospheric ClO$_4^-$ production rates, or higher ClO$_4^-$ production rates in pre-Pleistocene times.

Stable isotope analysis of Cl and O and radioactive isotope analysis of $^{36}$Cl in natural ClO$_4^-$ confirmed and extended initial data suggesting that indigenous ClO$_4^-$ sources in the southwestern U.S. show some isotopic variation by location and environment but remain isotopically distinct from synthetic and Atacama ClO$_4^-$ when all relevant isotope ratios are considered. ClO$_4^-$ concentration and isotope analysis was conducted in all five of the North American Great Lakes. The data showed average ClO$_4^-$ concentrations ranging from 0.05 to 0.13 µg/L (varying by lake) with concentrations being nearly constant with depth. Interestingly, the overall ranges of stable isotopic compositions of Great Lakes ClO$_4^-$ resemble those of indigenous natural ClO$_4^-$ measured in groundwaters of the western USA indicating a predominantly natural atmospheric source of ClO$_4^-$ in all of the lakes. Bomb-pulse $^{36}$Cl is largely retained in Lake Superior because of the 191-yr water residence time in the lake.

Several potential biological mechanisms of ClO$_4^-$ generation were evaluated to determine if any could be a secondary source of this anion in the environment and to help explain the isotopic characteristics and variation in some natural ClO$_4^-$ samples. Bacterial production of ClO$_4^-$ was assessed using (1) various nitrifying cultures and enrichments that oxidize NH$_4^+$ to NO$_3^-$; (2) natural haloperoxidase enzymes that are known to oxidize Cl$^-$ to hypochlorous acid (HClO) and potentially to ClO$_4^-$ (possibly via additional photochemical or biological reactions); and (3) organisms capable of oxidizing sulfite or phosphite. A variety of experiments were conducted with Cl$^-$ or ClO$_x$ precursors in the presence of different enzymes, organisms, and conditions as summarized above. While some ClO$_4^-$ generation was initially indicated via haloperoxidase enzymes in the presence of UV light, this result was not consistent and is unlikely to account for
significant ClO$_4^-$ production. The other organisms and processes evaluated did not result in ClO$_4^-$ formation.

A variety of plant species were also evaluated for their potential to accumulate and even generate ClO$_4^-$ via O$_3$-mediated processes. Plants, particularly in arid environments, may contain abundant Cl$^-$ 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 Cl$^-$ in plants. This was evaluated as a potentially novel source of ClO$_4^-$ in the environment.

A broad range of crop species was observed to accumulate ClO$_4^-$ from growth media, and these species differed widely in their bioconcentration of the anion. 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, there was no evidence that exposure of leaves to ambient O$_3$ or at significantly elevated O$_3$ induced any increase in tissue contents of ClO$_4^-$. The results indicate that O$_3$ does not lead to increased phyto-accumulation or plant biosynthesis of ClO$_4^-$.

The impact of plant accumulation of ClO$_4^-$ on Cl and O stable isotope values was also evaluated in both hydroponic laboratory studies and field crops grown in different parts of the U.S. In hydroponic studies with snap beans, no substantial differences were observed in the $\delta^{37}$Cl, $\delta^{18}$O, or $\Delta^{17}$O values of ClO$_4^-$ between the growth solutions and leaf extracts. In contrast to ClO$_4^-$, $\delta^{15}$N of NO$_3^-$ in plant tissue was fractionated substantially (~10-20‰). The $\epsilon^{15}$N/$\epsilon^{18}$O ratios of 1.04 – 1.07 support previous experimental studies showing similar ratios via assimilatory nitrate reductase. The data indicate that plants do not metabolize and assimilate ClO$_4^-$ similarly to NO$_3^-$. Similar to hydroponically grown plants, field grown plants exposed to environmentally relevant ClO$_4^-$ concentrations also did not appear to affect foliar ClO$_4^-$ isotopic composition. ClO$_4^-$ extracted from snap beans grown in Raleigh, NC varied somewhat in isotopic composition between two growing seasons based presumably on the source of irrigation water, but the stable isotope data clearly showed a significant component of Atacama-type ClO$_4^-$ from past fertilizer use which was also the predominant source in local groundwater based on isotopic analysis. Commercial spinach was also extracted and analyzed for ClO$_4^-$ stable isotopes. The spinach had an isotopic composition similar to that of indigenous natural ClO$_4^-$ from the Southern High Plains of West Texas and New Mexico as well as the North American Great Lakes. This result could indicate the spinach was exposed to natural ClO$_4^-$ with a similar isotopic composition in soil or irrigation water in one or both of the potential source areas of the spinach (Arizona and southwestern California). Although this spinach that was extracted represents only one composite sample, these results combined with those of field bean and hydroponic studies suggest that it should be possible to evaluate the dominant source of ClO$_4^-$ (i.e., synthetic, Atacama, indigenous) in commercial produce and in other plant-based food products through
stable isotope analysis of plant accumulated ClO$_4^-$.

This finding is important because most of the exposure to ClO$_4^-$ in the U.S. population and likely the populations of many other countries is through ingestion of produce.

**Benefits:**
Overall, the results of this project have provided important new information on natural ClO$_4^-$ in the environment. Significant progress was made concerning potential mechanisms of its formation, isotopic characterization of natural ClO$_4^-$ sources in groundwater, lakes, soils and plants, and its worldwide occurrence and accumulation in arid and semi-arid environments. The data support previous studies showing that natural and synthetic ClO$_4^-$ can be differentiated by stable isotope methods, and suggest for the first time that the source(s) of ClO$_4^-$ in food crops may be determined by isotopic analysis of ClO$_4^-$ in plant tissue.
1.0 PROJECT BACKGROUND AND OBJECTIVES

1.1 Background

In the United States (U.S.), perchlorate (ClO₄⁻) is mainly used as an oxidant in the production of rocket propellants and missile fuel by the U.S. Department of Defense (DOD), the National Aeronautics and Space Administration (NASA), and other munitions facilities. These facilities, along with road safety flares, fireworks, blasting explosives, and electrolytic chlorine products, like hypochlorite (OCl⁻) and chlorate (ClO₃⁻), are major contributors to the ClO₄⁻ contamination of water supplies in North America (ITRC, 2005; Geosyntec, 2005; Dasgupta et al., 2006). California and Nevada are two states heavily impacted by the anthropogenic use of ClO₄⁻. A former chemical manufacturing factory outside of Las Vegas, Nevada is considered to be the source of surface water contamination in Lake Mead and the Colorado River, along with groundwater in the area (Hogue, 2003). These waters are used as a drinking water resource for communities in California, Nevada, and Arizona and are also used for irrigation of crops grown in those regions (Hogue, 2003). Because of the use of ClO₄⁻ contaminated waters like these for irrigation purposes, vegetation has also been found to contain the ClO₄⁻ anion and could thus be transferred to humans or animals that ingest these crops (Hogue, 2003; Jackson et al., 2005) Along with edible vegetation, ClO₄⁻ has also been detected in milk, wine, beer, and vitamins (Jackson et al., 2005; Kirk et al., 2003; El Aribi et al., 2006; Snyder et al., 2006).

Studies conducted over the past few decades reveal that ClO₄⁻ occurrence is not limited to anthropogenic production or use. The oldest, most widely known natural source of ClO₄⁻ in the environment is the Atacama Desert in northern Chile. The Atacama Desert is one of the most arid places on Earth and contains caliche ores rich in nitrate (NO₃⁻) salts, which also contain ClO₄⁻ (Ericksen, 1983). Since the 1830s, the nitrate deposits have been used extensively as a nitrate source in fertilizers in many parts of the world (Ericksen, 1983). In more recent years, ClO₄⁻ of atmospheric origin has also been found in the groundwater of New Mexico and northwest Texas, rainwater across the U.S., and various unsaturated zones of the southwest U.S (Plummer et al., 2006; Rajagopalan et al., 2006, 2009; Rao et al., 2007; Jackson et al., 2010, 2016). The extent of naturally occurring ClO₄⁻ is not exclusive to North America, but has also been reported in high concentrations in the dry valleys of Antarctica and most interestingly in the soil of the planet Mars (Kounaves et al., 2010; Hecht et al., 2009).

The presence of ClO₄⁻ in produce and drinking water has created a public health concern and has further sparked debate on the importance of its removal from contaminated sites. Ingestion of the contaminant inhibits the uptake of iodide by the sodium-iodide symporter of the human thyroid, ultimately depressing the production of key thyroid hormones in humans (Greer et al., 2002). Because the thyroid gland is responsible for the neurological development and growth of youth, particularly infants and fetuses, proper thyroid hormone function is an important factor for proper maturity (Porterfield et al., 1994). Pregnant mothers and people who have iodine
deficiencies are more prone to be affected by ClO₄⁻ ingestion, with the deficiency in the mothers being more likely to affect the fetuses (Porterfield et al., 1994).

In 2005, the National Research Council (NRC) of the National Academies of Science presented a review outlining the health implications of ClO₄⁻ exposure to sensitive populations, such as those described above (National Academies, 2005). The Environmental Protection Agency (EPA) subsequently established a reference dose (RfD) for ClO₄⁻ of 0.7µg per kg body weight per day (USEPA, 2005). Although EPA has not officially set a national drinking water regulation for ClO₄⁻, the Agency has included the contaminant in the Contaminant Candidate List (CCL) for: 1998, 2005, and 2009. In February 2011 the EPA announced its intention to develop a drinking water standard for ClO₄⁻ (USEPA, 2011), but as of the publication of this document, this standard has not been set. Due to the lack of federal regulation, Massachusetts and California have established their own drinking water standard for ClO₄⁻ of 2 parts per billion (ppb; µg/L) and 6 µg/L, respectively. Other states have followed suit by setting guidance levels for ClO₄⁻ of their own in both drinking water and groundwater ranging from 1 to 18 µg/L (GAO, 2010). Federal regulatory guidelines for ClO₄⁻ are also important in establishing proper disposal techniques as well as lowering the cost of the cleanup of the contaminant by the facilities responsible for the discharge of the contaminant into the environment (GAO, 2010).

The chemical properties of ClO₄⁻ provide insight into its ubiquity and its potential for remediation. The ClO₄⁻ anion has a tetrahedral shape, with a central chlorine atom surrounded by four oxygen atoms. The chlorine atom in the ClO₄⁻ species has a charge of +7, an extremely high oxidation state, which makes it a desirable oxidizing agent (Brown et al., 2006). Perchlorate is meta-stable in aqueous systems and is especially resistant to reduction (Urbansky, 2002). High activation energy is required to break the kinetic barrier for ClO₄⁻ reduction, and thus reduction has been observed only under highly acidic conditions or by bacteria with specific enzymes (Brown et al., 2006; Urbansky, 2002; Coates and Achenbach, 2004). At ambient conditions, the ClO₄⁻ anion is unreactive and its low charge density inhibits formation of insoluble complexes with metals, hence its persistence in natural waters (Urbansky, 2002).

At the initiation of this research in 2008, few published data existed concerning the overall stable isotope signatures of natural ClO₄⁻ in the United States and abroad (Bao and Gu, 2004; Böhlke et al., 2005; Sturchio et al., 2006). However, these limited data indicated that natural ClO₄⁻ present in the southwest US was isotopically distinct from that found in the Atacama Desert, and that natural ClO₄⁻ from both areas was distinct from synthetic ClO₄⁻. These data were obtained mainly via ESTCP Project ER-200509, whose primary objective was the development and validation of stable isotope methods for chlorine (³⁷Cl and ³⁵Cl) and oxygen (¹⁸O, ¹⁷O, and ¹⁶O) in ClO₄⁻ (Böhlke et al., 2005, 2009; Sturchio et al., 2006, 2011, 2014; Hatzinger et al., 2011, 2013). Characterization of radioactive ³⁶Cl in ClO₄⁻ was also accomplished during that project (Sturchio et al., 2009). In conjunction with additional sampling and analysis conducted during the present project (see details in Section 2.1), the isotopic characteristics of natural ClO₄⁻
(Atacama and U.S. indigenous) were more clearly defined and compared to synthetic sources. The isotopic characteristics of these different types of ClO$_4^-$ are illustrated in Figure 1.1.1 and 1.1.2 and summarized as follows (from Hatzinger et al., 2013):

- Synthetic ClO$_4^-$ produced by electrochemical reaction is characterized by (1) a mean $\delta^{37}\text{Cl}$ value (with respect to Standard Mean Ocean Chloride; SMOC) of 0.6 ‰ and relatively narrow range of variation (-3.1 to +1.6 ‰), (2) more variable $\delta^{18}\text{O}$ values (with respect to Vienna Standard Mean Ocean Water; VSMOW) ranging from -24.8 to -12.5 ‰, and (3) $\Delta^{17}\text{O}$ values near 0 ‰, consistent with mass-dependent isotopic fractionation of O during ClO$_4^-$ synthesis. This material also is characterized by low $^{36}\text{Cl}/\text{Cl}$ ratios ($^{36}\text{Cl}$ mole fractions) of $1 \times 10^{-15}$ to $40 \times 10^{-15}$.

- Natural ClO$_4^-$ from caliche deposits in the Atacama Desert of Chile, and in nitrate fertilizers derived from this material, has reported $\delta^{37}\text{Cl}$ values ranging from -14.5 ‰ to -11.8 ‰, with a mean value more than 10 ‰ lower than that of synthetic ClO$_4^-$. Reported $\delta^{18}\text{O}$ values of Atacama ClO$_4^-$ (-24.8 ‰ to -4.2 ‰) exhibit substantial overlap with the $\delta^{18}\text{O}$ values of synthetic ClO$_4^-$, but the Atacama ClO$_4^-$ is characterized by substantially elevated values of $\Delta^{17}\text{O}$ (+4.2 to +9.6 ‰), indicating that non-mass-dependent isotope effects or precursors contributed to its formation, most likely during atmospheric generation. This natural ClO$_4^-$ has higher $^{36}\text{Cl}/\text{Cl}$ ratios ($22 \times 10^{-15}$ to $590 \times 10^{-15}$) compared to synthetic ClO$_4^-$.  

- Natural ClO$_4^-$ from the southwestern U.S. varies somewhat by location and environment. Samples collected from a large area of the Southern High Plains (SHP) and the Middle Rio Grande Basin (MRGB) of Texas and New Mexico are similar isotopically, with $\delta^{37}\text{Cl}$ values ranging from +3.1 to +5.0 ‰, $\delta^{18}\text{O}$ values ranging from +0.6 to +3.8 ‰, and $\Delta^{17}\text{O}$ values ranging from +0.3 to +1.3 ‰. Samples collected from the arid Umatilla Basin (LUB) of northeastern OR were similar isotopically to the SHP and MRGB samples, except that their $\Delta^{17}\text{O}$ values were somewhat higher (+1.7 to +2.9 ‰). The data indicate that indigenous natural ClO$_4^-$ in the western US (represented by samples from the SHP, MRGB, and LUB) is distinguishable from both Chilean ClO$_4^-$ and synthetic ClO$_4^-$ when all relevant stable isotope ratios are considered.

- Natural ClO$_4^-$ samples from caliche deposits in and around Death Valley, California have lower $\delta^{37}\text{Cl}$ values (-0.8 to -3.7 ‰) and much higher $\Delta^{17}\text{O}$ values (+8.6 to +18.4 ‰) compared to the SHP, MRGB, and LUB samples. Interestingly, however, all of the SHP, MRGB, LUB, and Death Valley samples analyzed to date are characterized by substantially elevated $^{36}\text{Cl}/\text{Cl}$ values ($3130 \times 10^{-15}$ to $28,800 \times 10^{-15}$) compared to those of synthetic or Chilean ClO$_4^-$. Overall, the SHP, LUB, MRGB, and Death Valley samples can be considered together as U.S. indigenous sources and, even though there are substantial ranges in the individual isotope ratios, this indigenous grouping is isotopically distinct from synthetic and Chilean ClO$_4^-$ when all relevant isotope ratios are considered.
Figure 1.1.1. Comparison of $\delta^{37}$Cl versus $\delta^{18}$O (Plot A) and $\Delta^{17}$O versus $\delta^{18}$O (Plot B) in natural indigenous ClO$_4^-$ in the U.S., natural Chilean ClO$_4^-$, and synthetic ClO$_4^-$. The single value on Plot B labeled with an asterisk is from Bao and Gu (2004). The graphs are from Hatzinger et al. (2013).
Figure 1.1.2. Values of $^{36}\text{Cl}/\text{Cl}$ (mole fraction) versus $\delta^{37}\text{Cl}$ in representative samples of synthetic $\text{ClO}_4^-$ reagents and products, natural $\text{ClO}_4^-$ and $\text{Cl}^-$ extracted from soil and groundwater from the Atacama Desert, Chile, and natural $\text{ClO}_4^-$ extracted from groundwater and soil from the southwestern U.S. Figure from Hatzinger et al., 2013.
The apparent widespread occurrence of natural ClO$_4^-$ in the western U.S., and the differing isotopic characteristics between natural ClO$_4^-$ derived from the Atacama Desert and that derived from the U.S., as well as the differences among samples from different regions of the southwest U.S. have generated new questions regarding the overall distribution of natural ClO$_4^-$ worldwide, mechanisms involved in the natural generation of ClO$_4^-$ and how isotopic compositions of the anion may be altered by various biotic and abiotic processes (Böhlke et al., 2005; Sturchio et al., 2009; Bao and Gu, 2004; Hatzinger et al., 2013). The isotopic data suggest that different ClO$_4^-$ production pathways exist and/or that post-depositional exchanges occur in nature.

Although ClO$_4^-$ production pathways for O$_3$ and UV reacting with a number of Cl starting materials have been demonstrated (e.g., Rao et al., 2010; Wang et al., 2011; Kang et al., 2008, 2009), it is still unclear which mechanism(s) or reactants are most significant in the environment. One way to approach this issue is to evaluate the stable isotopic composition of ClO$_4^-$ produced by these mechanisms and their associated starting materials in order to compare with the known natural isotopic compositions. Secondly, there are several common enzymatic oxidation reactions in nature (e.g., nitrification, hypochlorous acid generation from chloride, sulfur oxidation) that suggest the possibility of a biological mechanism for ClO$_4^-$ generation, which could help explain the isotopic variation in some of the natural ClO$_4^-$ samples. Furthermore, because there is a high potential for plants to uptake, accumulate, and possibly produce ClO$_4^-$, there is a need to examine ClO$_4^-$ interactions in plants and isotopic changes that may occur. No extensive research has been conducted to determine if real world plant samples grown under similar conditions all exhibit similar ClO$_4^-$ isotopic compositions or if ClO$_4^-$ isotopic compositions vary significantly between species. In addition, little research has examined the ability for plant uptake and transformation processes to fractionate ClO$_4^-$. 

The core objectives and associated major tasks associated with this project address the significant questions posed above. These objectives are described in detail in Section 1.2.
1.2 Research Objectives and Associated Major Tasks

**Objective 1.** Evaluate ClO$_4^-$ distribution, significant environmental ClO$_4^-$ production mechanisms and the stable isotopic signature of the produced ClO$_4^-$. 

**Task 1. Occurrence and isotopic signature of natural ClO$_4^-$.**

At the initiation of this project, there were relatively few data concerning the stable isotope signatures of natural ClO$_4^-$, most of which were gained through ESTCP Project ER-200509 (Hatzinger et al., 2013). During the current project, additional samples were collected and analyzed from various locations, including the Atacama Desert of Chile, the southwest US, and the US Great Lakes, among others. These data are reported herein.

**Task 2. Occurrence of natural ClO$_4^-$ and relationship with NO$_3^-$ and other anions in arid and semi-arid environments worldwide.**

Samples of soil and groundwater were collected from various arid and semi-arid locations around the world. Correlations between concentrations of ClO$_4^-$ and other co-occurring anions, including NO$_3^-$, Cl$^-$, SO$_4^{2-}$ and ClO$_3^-$ were evaluated. These data are important for understanding the origins, relationships, and environmental fate of these different species.

**Task 3. Determine the isotopic signature of ClO$_4^-$ produced by O$_3$ and UV-driven oxidation of relevant Cl species.**

A variety of chemical reactions and precursors could theoretically account for natural ClO$_4^-$ in the environment. Currently available isotopic data for natural ClO$_4^-$ indicate that multiple mechanisms may be producing natural ClO$_4^-$ and contributing to its broad distribution. However, the actual formation mechanism(s) of ClO$_4^-$ from various precursors are largely uncharacterized. The oxidation of chlorine and/or oxychlorine species to ClO$_4^-$ most likely results from reaction(s) with O$_3$ and/or another UV-driven photochemical process. One key objective of this research was to evaluate each potential reaction with respect to O$_3$ or UV-produced radicals to better understand likely mechanisms of formation of natural ClO$_4^-$ in the environment.

The isotopic composition of each product and initial reactant (e.g. O$_3$, H$_2$O, Cl, OCl$^-$, ClO$_2^-$, ClO$_3^-$, and ClO$_4^-$) in studies of UV and O$_3$ generation of ClO$_4^-$ was characterized to the extent possible. This entailed a series of purification steps followed by isotopic analysis. This information allowed evaluation of mechanistically correct formation pathway(s) of ClO$_4^-$ consistent with isotopic signatures of reactants and products as well as new constraints on the likely formation mechanisms of natural ClO$_4^-$. 
Task 4. **Evaluate the potential for microbial production of ClO$_4^-$**.

One potential production mechanism for natural ClO$_4^-$ that has not been previously evaluated is microbiological generation. Nitrifying bacteria generate NO$_3^-$ through the oxidation of NH$_4$ (e.g., *Nitrosomonas* spp.) and NO$_2^-$ (e.g., *Nitrobacter* spp.) (Paerl, 1997). During this well-studied process, NH$_4$ serves as a microbial electron donor and oxygen as an electron acceptor (Capone, 1997). There are also anaerobic oxidation processes by which NO$_3^-$ is biologically generated from reduced nitrogen species. Moreover, a class of widely occurring haloperoxidase enzymes produced by specific fungi, algae, and bacteria are known to oxidize Cl$^-$ to hypochlorous acid (HClO) (Griffin, 1991; Vilter, 1995). This could represent an initial biological step in the production of natural ClO$_4^-$ in some environments (possibly followed by additional photochemical or biological reactions). During this task, we evaluated whether microbial generation of ClO$_4^-$ (as well as HClO, ClO$_2^-$, and ClO$_3^-$) is possible in aerobic environments using pure cultures, pure enzymes and microbial enrichments.

**Objective 2. Evaluate the role of plants in production of ClO$_4^-$ and their impact on the isotopic signature of natural ClO$_4^-$**.

Task 5. **Evaluate the role of ozone in ClO$_4^-$ accumulation in plants**.

Recent reports indicate that a substantial percentage of total exposure may be linked to ingestion of food, mainly leafy vegetables, containing ClO$_4^-$ (e.g., USFDA, 2008; Blount et al., 2007). The ClO$_4^-$ concentrations in plant leaves and other tissues may reflect a variety of factors, including climatic conditions and species-specific physiological parameters. Sensitivity to ozone (O$_3$) may be particularly important in determining ClO$_4^-$ levels in plants. Tropospheric O$_3$ is a common air pollutant that has adverse effects on the growth of natural vegetation and agricultural crops worldwide (Burkey et al., 2002). Ambient ozone concentrations in the eastern United States range from 30-50 µg/L with episodes that may exceed 100 µg/L (Patterson et al., 2000). Ozone and associated reactive oxygen species react with all plant surfaces, but most of the O$_3$ damage is caused after these species enter the stomata (Heber et al., 1995). Upon passing the stomata, O$_3$ enters the apoplastic space of internal leaf tissues. It is within this apoplastic space that plant oxidative defense typically occurs. Some plants are more sensitive to O$_3$ than others. When plant oxidative defense capabilities are overwhelmed, visible foliar damage such as bronzing, flecking, and chlorosis can occur.

As natural ClO$_4^-$ could be produced from surface oxidation reactions of Cl$^-$ or oxychlorine species with O$_3$ (Kang et al., 2008), it is possible that ClO$_4^-$ may be directly produced in plant material from interaction of ambient O$_3$ with Cl$^-$, especially in certain O$_3$ sensitive plants that do not have active defense systems. In addition, plants have the capacity to process (uptake and re-release) a significant portion of natural ClO$_4^-$ even in arid climates. The isotopic composition of ClO$_4^-$ present within plants, and changes thereof due to processing have not yet been evaluated.
Therefore, it is important to understand:

1. If plants contribute to the natural ClO$_4^-$ pool by acting as platforms for oxidation of Cl$^-$ by O$_3$;

2. If plants can alter the isotopic signature of ClO$_4^-$ during uptake or internal processing;

During this task, studies were conducted with a variety of different plants at atmospheric and elevated O$_3$ concentrations to determine species variability and to assess whether plant production of ClO$_4^-$ with O$_3$ as a reactant could be substantiated. Task 6 addresses the question concerning alteration of the isotopic signature of O and Cl in plant ClO$_4^-$ after uptake.

**Task 6. Ion selectivity and isotope effects during plant uptake and accumulation of ClO$_4^-$ and NO$_3^-$.**

A primary objective of this task was to investigate the potential for plants to alter the stable isotopic composition of ClO$_4^-$ either during uptake or due to transformation or exchange within the plant. This was accomplished by comparing the stable isotopic compositions of ClO$_4^-$ accumulated in hydroponically grown snap bean plants (*Phaseolus vulgaris L.*) with those of the starting reference materials and growth solutions. Results for NO$_3^-$ uptake, assimilation, accumulation, and isotope effects were evaluated in the same experiments. Studies were also conducted with field-grown snap bean crops in Long Island, NY and Raleigh, NC to determine the isotopic signature of Cl and O in ClO$_4^-$ in these crops and whether sources (e.g., synthetic vs Atacama) could be distinguished from plant-derived ClO$_4^-$. Finally, a commercially obtained spinach sample that was conventionally grown was extracted and the ClO$_4^-$ obtained was analyzed for Cl and O isotopes. These isotope data were compared to those from potential ClO$_4^-$ perchlorate sources.
2.0 PROJECT TASKS

2.1 Occurrence and Isotopic Signature of Natural ClO$_4^-$ in Soils and Groundwater in the U.S.

2.1.1 Background: Natural Perchlorate and Nitrate

During the past decade, it has become apparent that ClO$_4^-$ is much more widely distributed in the environment than previously thought, and that a variety of natural and anthropogenic sources may contribute to its ubiquity. Major documented sources of ClO$_4^-$ distributed in the environment by human activities include: 1) electrochemically produced salts used as oxidants in solid rockets, air-bags, fireworks, flares, munitions and other industrial products; 2) hypochlorite and chlorate salts, which contain ClO$_4^-$ as a minor constituent; and 3) natural NO$_3^-$-rich caliche salt deposits containing ClO$_4^-$ from the Atacama Desert in Chile, which have been imported to the U.S. and elsewhere, primarily for use as fertilizer (Dasgupta et al., 2006; Aziz et al., 2006).

Natural ClO$_4^-$ that is unrelated to the Atacama source has now been detected widely in groundwater and soils in the southwestern United States (U.S.). This “indigenous” ClO$_4^-$ has been described in groundwater beneath a large area (53,000 km$^2$) of the southern High Plains (SHP) of Texas and New Mexico (Rajagopalan et al., 2006), in groundwater of Holocene and Pleistocene age in the Middle Rio Grande Basin (MRGB) in New Mexico (Plummer et al., 2005), and in pre-modern (mainly Holocene) atmospherically deposited salt accumulations in the vadose zone throughout the arid southwestern U.S. (Rao et al., 2007). Perchlorate is also ubiquitous in precipitation (Rajagopalan et al., 2009) and was detected (>40 ng L$^{-1}$) in more than 55 % of groundwater samples in a national survey of wells presumed to be minimally impacted by human activities (Parker et al., 2008). Given the widespread occurrence and potential regulatory importance of natural ClO$_4^-$, its origins and distinguishing characteristics (e.g., isotopic composition) are receiving increased attention, but remain poorly understood. Additional data are required to establish the isotopic characteristics and origin (or origins) of natural ClO$_4^-$ in the diverse environments in which it has been identified.

Stable isotope ratio analysis of Cl and O in ClO$_4^-$ has been used to distinguish anthropogenic ClO$_4^-$ from Atacama-derived natural ClO$_4^-$ in source materials and groundwaters (Bao and Gu, 2004; Böhlke et al., 2005, 2009; Sturchio et al., 2006). Electrochemically produced ClO$_4^-$ has relatively well-constrained $\delta^{37}$Cl values (-3 to +2 ‰), but more variable $\delta^{18}$O values (-25 to -13 ‰) likely reflecting variations in the source water and fraction of O lost during production, and $\Delta^{17}$O values that are consistent with mass-dependent fractionation of O isotopes ($\Delta^{17}$O = 0.0 ± 0.1 ‰) (Bao and Gu, 2004; Böhlke et al., 2005, 2009; Sturchio et al., 2006). In contrast, Atacama ClO$_4^-$ from saline caliche deposits and imported Chilean nitrate fertilizers has isotopic compositions ($\delta^{37}$Cl = -15 to -12 ‰; $\delta^{18}$O = -25 to -3 ‰ and $\Delta^{17}$O = +4 to +11 ‰) that are distinct from those of synthetic perchlorate. Elevated $\Delta^{17}$O values in ClO$_4^-$ from the Atacama Desert have been interpreted as evidence that ClO$_4^-$ were formed in part by reactions involving
ozone (O₃) in the atmosphere, as atmospheric O₃ is known to be ¹⁷O-enriched with measured and modeled Δ¹⁷O values of ~ + 30 to 40‰ (Bao and Gu, 2004; Johnson et al., 2000). Moreover, concentrations of cosmogenic ³⁶Cl are consistent with an upper atmospheric origin for natural ClO₄⁻ (Sturchio et al., 2009). Reported kinetic isotope effects accompanying biological reduction of ClO₄⁻ alter its isotopic composition in a predictable manner (Sturchio et al., 2003, 2007; Hatzinger et al., 2009) that does not obscure distinctions between synthetic and Atacama ClO₄⁻ when all relevant isotope ratios are considered (i.e., δ³⁷Cl, δ¹⁸O, and Δ¹⁷O).

At the initiation of this project, almost no information was available regarding the stable isotopic composition of natural ClO₄⁻ indigenous to the U.S. Two samples from groundwater wells in the SHP had reported isotopic compositions (δ³⁷Cl = +6.2, +5.1‰; δ¹⁸O = +4.7, +2.5‰; Δ¹⁷O = +0.4, +0.5‰) that were different from those of both anthropogenic and Atacama ClO₄⁻ (Sturchio et al., 2006). These data indicated that the SHP groundwater ClO₄⁻ was either a mixture of biologically fractionated, electrochemically produced ClO₄⁻ and a much smaller amount of Atacama-like ClO₄⁻, or that it represented an isotopically distinct type of natural ClO₄⁻. As a result of recent studies on the distribution and potential sources of ClO₄⁻ in the SHP (Rajagopalan et al., 2006), and rapidly increasing detection of trace ClO₄⁻ in soils (Rao et al., 2007), groundwater (Plummer et al., 2005; Parker et al., 2008), and precipitation (Rajagopalan et al., 2009) throughout the U.S., the latter explanation now appears more likely, but additional ClO₄⁻ isotope data are required to confirm this hypothesis and expand the database to other indigenous ClO₄⁻ occurrences. In addition, because ClO₄⁻ and NO₃⁻ typically coexist in terrestrial environments, relations between NO₃⁻ and ClO₄⁻ isotopes can provide important constraints on their sources and transport.

The purpose of this task was to develop data showing the variation in the isotopic composition of natural ClO₄⁻ indigenous to the southwestern U.S. Samples were collected from natural occurrences in soils and groundwater from the SHP of Texas and New Mexico and the MRGB in New Mexico; unsaturated sub-soil from the SHP of Texas; and surficial NO₃⁻-rich caliche deposits from the Mojave Desert near Death Valley, CA. These data were combined with other chemical and isotopic data to evaluate environmental factors responsible for ClO₄⁻ distribution and isotopic characteristics, including the origin and isotopic composition of co-occurring NO₃⁻. These studies were conducted in conjunction with ESTCP Project ER-200509.

2.1.2 Materials and Methods

2.1.2.1 Sampling Locations
Samples for ClO₄⁻ stable isotope ratio analysis were obtained from groundwater, unsaturated sub-soils, and caliche-type saline mineral deposits within the southwestern U.S. (Figure 2.1.1). These sites were selected because previous studies indicate that they represent natural occurrences. Additional samples were obtained from the Atacama Desert including one groundwater sample and 5 soil/caliche samples (Table 2.1.1 and 2.1.2).
Groundwater ClO$_4^-$ samples were obtained from the SHP (including one sample from the adjacent rolling plains) of western Texas and eastern New Mexico (n=8) and from the MRGB of central New Mexico (n=2) (Table 2.1.2). The SHP wells were at 5 distinct sites, with two wells (MW2 and MW3) installed at the same location but screened at different intervals. These wells were sampled in duplicate (MW2A,B and MW3A,B) (Figure 2.1.1). A single sample (SHP-V) was obtained from a natural subsurface accumulation of salts within unsaturated sub-soils at the Range Ecology Research Site at Texas Tech University. This site is a 142 ha section of land that has been used to study numerous aspects of range ecology but has not been irrigated or subjected to other surface activity that would impact the presence of ClO$_4^-$. Lastly, ClO$_4^-$ was obtained from near-surface caliche-type salt deposits on clay hills at four locations in the Death Valley region of the Mojave Desert, CA (Figure 2.1.1). Clay-hills caliche salts in this area were studied previously because of their unusually high NO$_3^-$ concentrations, which resemble those in the Atacama Desert (Böhlke et al., 1997; Ericksen et al., 1988; Noble, L.F. 1931; Michalski et al., 2004).

**Figure 2.1.1. Sample locations for natural perchlorate.** Surface deposits were collected from Death Valley, CA (red box), groundwater from the Rio Grande Basin of NM (green box) and vadose soils and groundwater from the SHP of Texas (blue box).
2.1.2.1 Sample Collection

ClO$_4^-$ in groundwater was collected by pumping water from each well through columns containing ClO$_4^-$-selective anion-exchange resin (Purolite A-530E, Purolite Co., Bala Cynwyd, PA). Groundwater from wells MW3, MW2, BW2, RR8, and RR16 was pumped through resin columns in the field at flow rates ranging from ~ 0.1 to 2 L min$^{-1}$. For the remaining wells, water was pumped into clean polyethylene drums (208-L capacity), which were then transported to Texas Tech University where the water was passed through ion-exchange columns, as described above. The total volume of water pumped through each column varied with ClO$_4^-$ concentration, with the final objective being to extract at least 5 mg of ClO$_4^-$ for purification and isotopic analysis. Groundwater was also collected for major anions, other isotopic analyses (NO$_3^-$, SO$_4^{2-}$, H$_2$O), major dissolved gases, and environmental tracers including $^3$H, $^3$He, SF$_6$, and chlorofluorocarbons (CFCs) (Table 2.1.2).

Perchlorate dispersed in the unsaturated zone (SHP-V) was collected by leaching soluble salts from the sub-soil and then passing the leachate through a resin column as described above for groundwater. Initially, depth-dependent samples were obtained by hand auger to evaluate the vertical distribution of salts in the unsaturated zone. Based on these data, sub-soil from approximately 2 to 4 m (depth range of maximum ClO$_4^-$ concentration) was collected using a back hoe and placed on a tarp. Salts were extracted by mixing batches of sub-soil (40 to 60 L) and water (80 L) in a pre-cleaned cement mixer for ~10 minutes to form a slurry. Tap water from Lubbock, Texas was used for the extraction. Prior to use, this tap water was passed through a large column (~1,000 cm$^3$) packed with Purolite A-530E resin to reduce ClO$_4^-$ to < 0.05 µg L$^{-1}$. After mixing, the slurry was allowed to settle for several hours, and then the water was decanted into polyethylene drums. The slurry in the drums was allowed to settle further overnight, after which the supernatant was pumped through a sediment pre-filter (50-µM pore-size; General Electric Co., Trevose, PA) and then through a resin column. Influent and effluent samples were taken routinely to determine the concentration of ClO$_4^-$ applied to the column and the efficiency of perchlorate removal by the column. A total of ~5,600 kg of soil and ~3,000 L of water were processed for the extraction.

The specific location and depth of the caliche-type salt accumulations collected from Death Valley were based on the NO$_3^-$ content of the deposits, which were determined by field testing. NO$_3^-$ was used as an indicator of ClO$_4^-$ based on previous data. Bulk samples (20 to 50 kg) from each location were shipped to Texas Tech University and portions of these samples were leached using ClO$_4^-$-free (< 0.05 µg L$^{-1}$) distilled de-ionized water. The samples were sequentially extracted three times with a ~1:5 solid to water mass ratio each time. The ClO$_4^-$ dissolved in the supernatant of these extracts was combined and collected on resin columns as described above. The concentrations of soluble salts in the bulk solids were estimated after drying and weighing the leached material after extraction. Aliquots of the leachate solutions were filtered and stored for chemical and isotopic analysis of solutes including ClO$_4^-$, NO$_3^-$, SO$_4^{2-}$, and Cl$^-$. 

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2.1.2.3 Methods for Separating and Purifying Perchlorate for Isotopic Analysis
The perchlorate samples were purified according to the methods described in Hatzinger et al., (2011). In summary, the resin from each IX column (from soils or groundwater) was dispersed in deionized water or 4M HCl in the laboratory, ultrasonically cleaned, and then repacked into a column for elution. Prior to elution, the resin was flushed with three to five bed volumes (BV) of 4M HCl to remove anions (NO₃⁻, SO₄²⁻, HCO₃⁻, organic anions, etc.) and other impurities. Then ClO₄⁻ was eluted with 3-5 BV of a mixed solution of 1M FeCl₃ and 4M HCl, and is usually concentrated in <0.5 BV of the eluent solution. To remove Fe³⁺ from the eluent solution, neutralization with NaOH solution to pH 9-10, followed by settling and centrifugation was used to remove Fe precipitates. The resulting clear solution was then reduced by evaporation to a smaller volume (0.5-10 mL) for analysis of ClO₄⁻ concentration and other anionic impurities by Raman spectroscopy and/or ion chromatography. Hydrogen peroxide may be added during evaporation to oxidize organics. If necessary, a second stage of purification using a smaller A-530E column or solid-phase extractant was used to remove residual ions and impurities to achieve the desired purity of ClO₄⁻ as described by Hatzinger et al. (2011). Finally, the purified and concentrated ClO₄⁻ in solution was crystallized by the addition of CsCl or CsOH to cause supersaturation and precipitation of CsClO₄. The CsClO₄ precipitate was then washed with 90% MeOH and air dried prior to isotopic analysis by isotope-ratio mass spectrometry. Purity of final CsClO₄ crystals was verified by micro-Raman spectroscopy. Additional details pertaining to the use of A-530E resin for ClO₄⁻ extraction and purification are provided elsewhere (Hatzinger et al., 2011; Gu et al., 2011).

2.1.2.4 Sample Analysis
Purified ClO₄⁻ in the form of CsClO₄ was shipped to the USGS laboratory in Reston Virginia for analysis of δ¹⁸O and Δ¹⁷O on O₂ produced by decomposition. Chloride residue from the decomposed ClO₄⁻ was analyzed for δ³⁷Cl at the University of Illinois at Chicago as described below. ClO₄⁻ concentrations were measured by sequential IC-MS/MS with a method detection limit of 0.05 µg L⁻¹ (Rao et al., 2007). Major anions (Cl⁻, NO₃⁻, SO₄²⁻, and Br⁻) were analyzed by ion chromatography following EPA Method 300.0. Major dissolved gases, and stable isotope ratios in NO₃⁻ and SO₄²⁻ (δ¹⁸O, δ¹⁵N, and δ³⁴S) were analyzed at the USGS in Reston (USGS, 2010). Δ¹⁷O analyses of NO₃⁻ were also performed at the USGS in Reston on O₂ produced by thermal decomposition of purified NO₃⁻ [see next section]. Tritium was analyzed by electrolytic enrichment and scintillation counting at the USGS in Menlo Park, CA. ¹⁴C was analyzed by accelerator mass spectrometry under contract to the USGS National Water-Quality Laboratory (Table 2.1.2 ).

2.1.2.5 Methods for Perchlorate Stable Isotope Analysis and Reporting
Tabulated and plotted values of δ¹⁸O, Δ¹⁷O, and δ³⁷Cl for ClO₄⁻ were determined by off-line sealed-tube decomposition and dual-inlet isotope-ratio mass spectrometry on O₂ (designated O₂-DIIRMS) and CH₃Cl, and the data were calibrated by analyzing ClO₄⁻ reference materials with
the samples as described elsewhere (Hatzinger et al., 2011; Böhlke et al., 2016). Reference values adopted provisionally for USGS37: $\delta^{37}\text{Cl} = +0.6$ ‰, $\delta^{18}\text{O} = -17.0$ ‰, and $\Delta^{17}\text{O} = 0.0$ ‰; and for USGS38: $\delta^{37}\text{Cl} = -87.2$ ‰, $\delta^{18}\text{O} = +52.4$ ‰, and $\Delta^{17}\text{O} = +73.3$ ‰. A subset of the samples were also analyzed for $\delta^{18}\text{O}$ by an alternative method involving high-temperature reaction with C to produce CO, with continuous-flow isotope-ratio mass spectrometry on the CO (designated CO-CFIRMS), calibrated using the same reference materials as above. For reagents and samples with relatively high original ClO$_4^-$ concentrations, $\delta^{18}\text{O}$ values determined by O$_2$-DIIRMS and CO-CFIRMS methods generally were indistinguishable. For some samples purified from low ClO$_4^-$ soils and groundwaters, however, the O$_2$-DIIRMS values tend to be slightly lower (commonly of the order of 0.5 to 1.0 ‰). These differences are not completely understood and may be due to trace contaminants in the samples that are most difficult to purify. Nevertheless, the analytical differences are small compared to the range of isotopic compositions reported for the different ClO$_4^-$ sources. Detailed descriptions of analytical interferences and calibrations are given by Böhlke et al. (2016).

2.1.2.6 Methods for Nitrate Stable Isotope Analysis

$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in NO$_3^-$ were measured by continuous-flow isotope-ratio mass spectrometry on N$_2$O produced from NO$_3^-$ by bacterial reduction (Sigman et al., 2001; Casciotti et al., 2002; Coplen et al., 2004). The data were calibrated by analyzing NO$_3^-$ isotope reference materials using calibration data in Böhlke et al. (2003). For USGS34, $\delta^{15}\text{N} = -1.8$ ‰ and $\delta^{18}\text{O} = -27.9$ ‰; for USGS32, $\delta^{15}\text{N} = 180.0$ ‰; for USGS35, $\delta^{18}\text{O} = +57.5$ ‰. For samples with elevated $\Delta^{17}\text{O}$ of NO$_3^-$, $\delta^{15}\text{N}$ values measured by the bacterial method using conventional normalization equations were adjusted downward to account for non-mass-dependent $\Delta^{17}\text{O}$ effects on the N$_2$O ion ratios, based on the measured $\Delta^{17}\text{O}$ values of the NO$_3^-$ (Sigman et al., 2001; Böhlke et al., 2003; Coplen et al., 2004). This adjustment to $\delta^{15}\text{N}$ was equal to 0 when $\Delta^{17}\text{O} = 0$ and -1.1 ‰ when $\Delta^{17}\text{O} = +21$ ‰.

$\Delta^{17}\text{O}$ in NO$_3^-$ was measured by dual-inlet isotope-ratio analysis of O$_2$ produced by off-line partial decomposition of AgNO$_3$ (Michalski et al., 2002). NO$_3^-$ was isolated from mixed salt solutions by trapping on large-volume AG1X8 ion-exchange resin columns, followed by gradual elution with 0.5 M KCl to separate anions (Hannon et al., 2008). The KCl-KNO$_3$ eluent was passed through AG-MP50 cation-exchange resin columns in the Ag form to remove Cl and exchange K for Ag, then freeze dried to produce AgNO$_3$ salt. The AgNO$_3$ was heated under vacuum at 520°C while connected to a 5Å mol-sieve trap cooled with liquid N$_2$ to collect O$_2$, which was then isolated and transferred to the mass spectrometer and analyzed against tank O$_2$. No adjustments were made to the $\Delta^{17}\text{O}$ data, as the measured $\Delta^{17}\text{O}$ values of NO$_3^-$ isotopic reference materials RSIL-N11 and USGS35 were indistinguishable from reported values of -0.2 and +21.1 ‰, respectively (Böhlke et al., 2003; Coplen et al., 2004).
2.1.3 Results and Discussion

2.1.3.1 Death Valley Caliche Salts

In the U.S., surficial NO$_3^-$-rich caliche deposits exist in the clay hills around the southern end of Death Valley within the Mojave Desert. These NO$_3^-$ deposits were first described in the early 1920s and are much smaller in both aerial extent and total mass than the Atacama deposits, although the NO$_3^-$ concentrations and NO$_3^-$/Cl$^-$ ratios locally are similar to those of the Atacama NO$_3^-$ ores (Böhlke et al., 1997; Ericksen et al., 1988; Noble, 1931; Noble et al., 1922). Isotopic analyses of the Death Valley NO$_3^-$ deposits indicate a large fraction of the NO$_3^-$ may be atmospheric in origin, whereas the remainder is presumed to have formed via microbial nitrification (Böhlke et al., 1997; Michalski et al., 2004). Similar processes were hypothesized to account for NO$_3^-$ in the Atacama NO$_3^-$ deposits based on isotopic analyses with a larger fraction due to atmospheric origin (Böhlke et al., 1997; Michalski et al., 2004). As part of the current study, new samples of NO$_3^-$ and ClO$_4^-$ from caliche salts and groundwater in the Atacama Desert were analyzed isotopically, confirming previous results (Figure 2.1.2; Table 2.1.3). Unlike the Atacama deposits, where the presence of ClO$_4^-$ is well established, ClO$_4^-$ has not previously been documented as a common constituent of the Death Valley deposits (Noble, 1931; Ericksen, 1981).

In the collected Death Valley caliche samples, ClO$_4^-$ concentrations ranged from 0.25 to 1.7 mg kg$^{-1}$, which are the highest reported ClO$_4^-$ concentrations in any natural material in North America, but still approximately 1-3 orders of magnitude lower than in the Atacama NO$_3^-$-rich caliches (Ericksen et al., 1981). Interestingly, the NO$_3^-$ content of the Death Valley caliche is similar to that of the Atacama caliche, or at most an order of magnitude lower (Figure 2.1.3; Table 2.1.3). Nitrate stable isotope ratios ($\delta^{18}O$, $\Delta^{17}O$, and $\delta^{15}N$) of the Death Valley caliche samples collected for this study overlap previously reported values for these deposits (Böhlke et al., 1997; Michalski et al., 2004) confirming a substantial atmospheric component of the NO$_3^-$ (Table 2.1.3, Figure 2.1.4).

Perchlorate stable isotopic compositions of the Death Valley caliches are distinct from those of previously reported sources (Figure 2.1.2; Table 2.1.3). The $\delta^{37}Cl$ values are higher than those of Atacama ClO$_4^-$ (including new data reported here) and generally lower than those of synthetic ClO$_4^-$ . The $\delta^{18}O$ values are higher than those of both Atacama and synthetic ClO$_4^-$ . However, the $\Delta^{17}O$ values of Death Valley caliche ClO$_4^-$ generally are similar to those of Atacama ClO$_4^-$ with the exception of the Zabriskie sample ($\Delta^{17}O = +18.4\%$), which has the highest $\Delta^{17}O$ value reported to date for ClO$_4^-$ (Figure 2.1.2). Combined data from Death Valley and Atacama indicate a positive correlation between $\Delta^{17}O$ and $\delta^{18}O$ in caliche ClO$_4^-$, with one end of the correlation line pointing toward the isotopic composition of atmospheric O$_3$ and the other end approaching the terrestrial mass-dependent fractionation line at a negative $\delta^{18}O$ value (Figure 2.1.3) (see also Bao and Gu, 2004). This pattern is similar to the one defined by NO$_3^-$ isotopic data, for which atmospheric and biogenic end-members have been proposed (Figure 2.1.4).
2.1.3.2 Middle Rio Grande Basin Groundwater

ClO$_4^-$ is present in Pleistocene and Holocene groundwater (0 to 28,000 years old) with minimal anthropogenic influence in the MRGB, New Mexico, at concentrations ranging from 0.12 to 1.8 µg L$^{-1}$ and with no systematic relation between groundwater age and ClO$_4^-$ concentration (Plummer et al., 2005). Wells sampled for the current study (RR8 and RR16; Table 2.1.2) are within the area of Pleistocene groundwater mapped previously as “northwestern recharge zone” and attributed to mountain-front recharge from low elevations around the southern part of the Jemez Mountains (Plummer et al., 2004). This water is relatively dilute, with high dissolved O$_2$, and major anion concentrations (Cl$^-$, Br$^-$, SO$_4^{2-}$) that may largely represent atmospheric fluxes with varying amounts of evapotranspiration (Plummer et al., 2004). The $\delta^{18}$O values of the NO$_3^-$ in these wells are much lower than those of atmospheric NO$_3^-$ and are consistent with values produced by nitrification in soils. The $\delta^{15}$N values of the NO$_3^-$ are higher than those of atmospheric N species, possibly indicating partial loss and isotope fractionation of N in soils prior to nitrification (McMahon et al., 2006; see Figure 2.1.4). Dissolved gas concentrations and NO$_3^-$ isotopes do not indicate denitrification in the saturated zone, hence it is also unlikely that ClO$_4^-$ was reduced in the saturated zone given the residual NO$_3^-$ in solution.

The sampled wells in this previously documented aquifer location had similar ClO$_4^-$ concentrations and ClO$_4^-$ isotopic compositions (Figure 2.1.2; Table 2.1.4). Estimated groundwater ages for both samples are greater than 10,000 yrs ($^3$H $\leq$ 0.3 TU and DIC $^{14}$C $<$12 pmc) (Table 2.1.4). Multiple lines of chemical, isotopic, and chronologic evidence indicate that this groundwater ClO$_4^-$ is natural in origin (Plummer et al., 2004, 2005; Table 2.1.2 and 2.1.4), yet the ClO$_4^-$ isotopic composition is distinct from those of the natural caliche-type occurrences, particularly with respect to the much lower MRGB $\Delta^{17}$O values (Figure 2.1.2). Instead; the MRGB ClO$_4^-$ is similar isotopically to ClO$_4^-$ from SHP groundwater (see next section and Böhlke et al., 2005).

2.1.3.3 Southern High Plains Groundwater

ClO$_4^-$ is present in groundwater (~ 0.1 to 200 µg L$^{-1}$) in at least 54 counties covering 155,000 km$^2$ in the SHP of Texas and New Mexico (Rajagopalan et al., 2004). The distribution and total mass of ClO$_4^-$ in SHP groundwater appear to preclude anthropogenic sources (e.g. Atacama nitrate fertilizer, chlorate defoliants, fireworks, explosives, or flares) (Rajagopalan et al., 2004). Rather, the SHP ClO$_4^-$ was interpreted to be natural and may represent wet and/or dry atmospheric deposition that accumulated in the unsaturated zone over millennial time scales and then was flushed to groundwater as widespread irrigation became common starting in the 1930s (Rajagopalan et al., 2004). Groundwater from wells evaluated in this study have apparent groundwater ages that range from modern (e.g., JYT-1 with 100 percent modern C and post-bomb $^3$H) to more than 10,000 years (e.g., MW3 with 34 percent modern C and $^3$H near the detection limit); however, there is evidence for mixing of old and young water in some cases (MW2 with low $^{14}$C and post-bomb $^3$H) (Table 2.1.4).
Samples for ClO$_4^-$ isotopic analysis were collected from groundwater at five sites in the SHP, including two previously sampled sites, spread across an area of ~40,000 km$^2$ (Figure 2.1.1). ClO$_4^-$ concentrations in these samples ranged from 1.8 to 200 $\mu$g L$^{-1}$ (Table 2.1.4). The new SHP ClO$_4^-$ samples all have similar isotopic compositions that are indistinguishable from those of the two SHP ClO$_4^-$ samples analyzed previously (Böhlke et al., 2005) (Figure 2.1.2; Table 2.1.4). Two hypotheses were advanced previously as possible explanations of ClO$_4^-$ isotope data in SHP groundwater: (1) it is a mixture of biologically degraded synthetic ClO$_4^-$ plus a small (~5%) amount of Atacama ClO$_4^-$; or (2) it is an isotopically distinct form of natural ClO$_4^-$ (Böhlke et al., 2005). Given the reported ubiquity of ClO$_4^-$ in the SHP groundwater and soils, its relatively homogeneous isotopic composition in samples with a wide range of concentrations, and its similarity to ClO$_4^-$ in old groundwater in the MRGB, it appears likely these data represent a major natural ClO$_4^-$ province that is isotopically different from the Atacama and Death Valley caliche ClO$_4^-$ occurrences (Figure 2.1.2). The alternative hypothesis (mixing of biodegraded synthetic perchlorate plus Atacama ClO$_4^-$) would require the unlikely circumstance of a consistent mixing proportion of synthetic and Atacama ClO$_4^-$, along with a constant isotopic shift due to biodegradation, over a wide geographical region.

NO$_3^-$ in the SHP groundwater generally had $\delta^{18}$O values consistent with biogenic sources. Two SHP NO$_3^-$ samples had low $\Delta^{17}$O values (+0.3 and +0.1 ‰ for MW2 and KJ1, respectively), also consistent with a predominantly biogenic source of the NO$_3^-$. Sample GW2 had relatively low O$_2$ concentration, high $\delta^{15}$N, and high $\delta^{18}$O, possibly indicating partial denitrification.

2.1.3.4 Southern High Plains Unsaturated Zone
To further test the hypothesis that ClO$_4^-$ in SHP groundwater was remobilized after having accumulated naturally in the unsaturated (vadose) zone, a sample of ClO$_4^-$ for isotopic analysis (SHP-V) was extracted from unsaturated sub-soil (2-4m) beneath undisturbed rangeland. High concentrations of disseminated salts peaking at depth in the unsaturated zone are common in the southwestern U.S. and have been interpreted as atmospheric salts accumulated largely during Holocene time since the last major wet climate period in this region (Rao et al., 2006; Phillips, 1994; Walvoord et al., 2002). The ClO$_4^-$ and Cl$^-$ concentrations in these accumulations are correlated ($r=0.59-0.99$) and the estimated mass of ClO$_4^-$ in the unsaturated zone (408±88 g ha$^{-1}$) is more than sufficient to account for the estimated mass of ClO$_4^-$ in SHP groundwater (Rao et al., 2006). The depth profiles of ClO$_4^-$ and Cl$^-$ at our sample site are similar to previously reported profiles in the SHP and elsewhere in the southwestern U.S., with an apparent concentration maximum at ~3-4 m depth and maximum Cl$^-$ and ClO$_4^-$ concentrations of 370 mg kg$^{-1}$ and 3.3 $\mu$g kg$^{-1}$, respectively (Figure 2.1.5). Our profile data are incomplete, as we did not sample below 4 m, yet the mass of Cl$^-$ above 4 m represents >5,000 years of accumulation based on a Cl$^-$ deposition rate of 157 mg/ha-year (Rao et al., 2004). The ClO$_4^-$ isotopic composition of this sample ($\delta^{37}$Cl$=$ +3.7 ‰; $\delta^{18}$O$= +2.1$ ‰; and $\Delta^{17}$O $= +0.8$ ‰) falls within the range of the SHP and MRGB groundwater samples (Figure 2.1.2). This sample therefore supports the
interpretation that widespread ClO$_4^-$ in groundwater throughout the SHP is of natural origin and has a characteristic isotopic composition distinct from those of Atacama and Death Valley caliches.
Table 2.1.1. Information about soil and caliche samples.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Location</th>
<th>Land Use</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Date Sampled</th>
<th>Sample Depth</th>
<th>Sample Description</th>
<th>Elevation (m)</th>
</tr>
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<tbody>
<tr>
<td>Confidence Hills 1</td>
<td>Death Valley, California, USA (Clay Hills)</td>
<td>National Park</td>
<td>N 35 50.350</td>
<td>W 116 35.256</td>
<td>2/16/2006</td>
<td>&lt;1 m</td>
<td>Salt-cemented salt and clay</td>
<td>4</td>
</tr>
<tr>
<td>Confidence Hills 2</td>
<td></td>
<td></td>
<td>N 35 50.372</td>
<td>W 116 35.47</td>
<td>1/25/2008</td>
<td>~10-30cm</td>
<td>Salt-cemented green and red clay with chunks of mixed salts</td>
<td>40</td>
</tr>
<tr>
<td>Saratoga Hills</td>
<td></td>
<td></td>
<td>N 35 40.050</td>
<td>W 116 28.197</td>
<td></td>
<td>~5-30cm</td>
<td>Salt-cemented red clay streaked with white veins</td>
<td>130</td>
</tr>
<tr>
<td>Bully Hill</td>
<td></td>
<td></td>
<td>N 35 47.647</td>
<td>W 116 12.346</td>
<td></td>
<td>~5-30cm</td>
<td>Salt-cemented green and red clay with chunks of mixed salts</td>
<td>390</td>
</tr>
<tr>
<td>Zabriskie</td>
<td>Bureau of Land Management</td>
<td></td>
<td>N 35 55.103</td>
<td>W 116 16.234</td>
<td></td>
<td>~5-30cm</td>
<td>Salt-cemented green and red clay with chunks of mixed salts</td>
<td>420</td>
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<tr>
<td>P1</td>
<td>Baquedano District, Chile</td>
<td>Surface NO₃⁻ mines</td>
<td>S23 11.863</td>
<td>W69 40.635</td>
<td>10/12/2007</td>
<td>&gt;6m</td>
<td>Salts in fractured andesite</td>
<td>1279</td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td></td>
<td>S23 11.863</td>
<td>W69 40.635</td>
<td></td>
<td>&gt;6m</td>
<td>Salts in fractured andesite</td>
<td>1279</td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td></td>
<td>S23 12.059</td>
<td>W69 40.205</td>
<td></td>
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</tr>
<tr>
<td>P4</td>
<td></td>
<td></td>
<td>S23 12.059</td>
<td>W69 40.205</td>
<td></td>
<td>50cm</td>
<td>NaNO₃ caliche</td>
<td>1304</td>
</tr>
<tr>
<td>GJ01</td>
<td>Chile</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>caliche</td>
<td>Unknown</td>
</tr>
<tr>
<td>UIC 24</td>
<td>Chile</td>
<td>Railway Cut</td>
<td>S23.16.53</td>
<td>W 69.46.14</td>
<td>11/03/2007</td>
<td>5m</td>
<td>NO₃⁻ vertical vein in regolith</td>
<td>1137</td>
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### Table 2.1.2. Well information with chemical and isotopic data for groundwater samples.

<table>
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<th>Well Name</th>
<th>Middle Rio Grande Basin</th>
<th>Southern High Plains</th>
</tr>
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<tr>
<td></td>
<td>RR8</td>
<td>RR16</td>
</tr>
<tr>
<td>Well Type</td>
<td>PSW ¹</td>
<td>PSW</td>
</tr>
<tr>
<td>County, State</td>
<td>Sandoval, NM</td>
<td>Sandoval, NM</td>
</tr>
<tr>
<td>Latitude (N)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Longitude (W)</td>
<td>-106.732</td>
<td>-106.693</td>
</tr>
<tr>
<td>Land Surface Elevation (m)</td>
<td>1776</td>
<td>1686</td>
</tr>
<tr>
<td>Mid-Screen Elevation (m)</td>
<td>1383</td>
<td>1257</td>
</tr>
<tr>
<td>Screen Elevation ± (m)</td>
<td>94</td>
<td>178</td>
</tr>
<tr>
<td>Water-Table Elevation (m)</td>
<td>1468</td>
<td>1488</td>
</tr>
<tr>
<td>Unsaturated-Zone Thickness (m)</td>
<td>308</td>
<td>198</td>
</tr>
<tr>
<td>Sample Date</td>
<td>9/12/2007</td>
<td>9/13/2007</td>
</tr>
<tr>
<td>Field parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (°C)</td>
<td>22.6</td>
<td>21.5</td>
</tr>
<tr>
<td>Specific Conductivity (µS/cm)</td>
<td>4709</td>
<td>4508</td>
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<td>Field O₂ (µmol/L) (±)</td>
<td>213</td>
<td>174</td>
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<tr>
<td>Field pH</td>
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<td>8.25</td>
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<tr>
<td>Water Chemistry</td>
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<td></td>
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<tr>
<td>TDS (g/L)</td>
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<td>F (µmol/L)</td>
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<td>58</td>
</tr>
<tr>
<td>Cl (µmol/L)</td>
<td>255</td>
<td>598</td>
</tr>
<tr>
<td>Br (µmol/L)</td>
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<td>2</td>
</tr>
<tr>
<td>NO₃ (µmol/L)</td>
<td>187</td>
<td>137</td>
</tr>
<tr>
<td>SO₄ (µmol/L)</td>
<td>669</td>
<td>1039</td>
</tr>
<tr>
<td>ClO₃ (µmol/L)</td>
<td>0.0067</td>
<td>0.0085</td>
</tr>
<tr>
<td>Alkalinity (µmol/L as HCO₃⁻)</td>
<td>4200</td>
<td>4000</td>
</tr>
<tr>
<td>Isotopes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O δ¹H (%)</td>
<td>-85.4</td>
<td>-96.8</td>
</tr>
<tr>
<td>H₂O δ¹⁸O (%)</td>
<td>-11.66</td>
<td>-12.90</td>
</tr>
<tr>
<td>H₂O δ¹³C (%)</td>
<td>-0.22 (0.27)</td>
<td>0.32 (0.22)</td>
</tr>
<tr>
<td>DIC δ¹³C (‰)</td>
<td>-7.7</td>
<td>-6.4</td>
</tr>
<tr>
<td>DIC δ¹⁴C (pmc)</td>
<td>11.60 (2.3)</td>
<td>5.90 (1.0)</td>
</tr>
<tr>
<td>DIC pmc Age (years)³</td>
<td>17300</td>
<td>22700</td>
</tr>
<tr>
<td>SO₄ ± δ¹⁸O (%)</td>
<td>-0.10 (2.0)</td>
<td>4.80 (2.2)</td>
</tr>
<tr>
<td>SO₄ ± δ³⁴S (%)</td>
<td>3.00 (2.0)</td>
<td>4.50 (1.1)</td>
</tr>
<tr>
<td>Dissolved Gases</td>
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<td></td>
</tr>
<tr>
<td>CH₄ (µmol/L) (±)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Ar (µmol/L) (±)</td>
<td>12.35 (0.02)</td>
<td>13.9 (0.03)</td>
</tr>
<tr>
<td>N₂ (µmol/L) (±)</td>
<td>497 (2)</td>
<td>534 (1)</td>
</tr>
<tr>
<td>Ar-N₂ equilibration T (°C)</td>
<td>19.2</td>
<td>16.3</td>
</tr>
<tr>
<td>Ar-N₂ excess air (ccSTP/L)</td>
<td>1.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>

¹ Available in PSW repository. ³ Calculations based on specific conditions.
Table 2.1.3. Selected chemical and isotopic data for caliche-type salts in unconsolidated surficial material from the Atacama Desert in Chile and the Death Valley clay hills region of the Mojave Desert in California, USA.

<table>
<thead>
<tr>
<th>Site</th>
<th>Non-soluble fraction</th>
<th>Concentration</th>
<th>ClO₄⁻ isotopes</th>
<th>NO₃⁻ isotopes</th>
<th>NO₂⁻ isotopes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>δ³⁷Cl⁰</td>
<td>δ¹⁸O⁰</td>
<td>Δ¹⁷O⁰</td>
</tr>
<tr>
<td>Death Valley</td>
<td>%</td>
<td>mg kg⁻¹</td>
<td>g kg⁻¹</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Confidence Hills 1</td>
<td>NA</td>
<td>0.25</td>
<td>320</td>
<td>1.8</td>
<td>72</td>
</tr>
<tr>
<td>Confidence Hills 2</td>
<td>49</td>
<td>0.85</td>
<td>180</td>
<td>5.5</td>
<td>100</td>
</tr>
<tr>
<td>Saratoga Hills</td>
<td>78</td>
<td>0.95</td>
<td>63</td>
<td>5.9</td>
<td>23</td>
</tr>
<tr>
<td>Bully Hill</td>
<td>62</td>
<td>0.82</td>
<td>80</td>
<td>28</td>
<td>6.5</td>
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<td>Zabriskie</td>
<td>64</td>
<td>1.7</td>
<td>140</td>
<td>4.4</td>
<td>39</td>
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<td></td>
<td>Atacama</td>
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<td></td>
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<td>243</td>
<td>80</td>
<td>12</td>
<td>57</td>
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<td>328</td>
<td>456</td>
<td>44</td>
<td>84</td>
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<td>51</td>
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<tr>
<td>GJ01</td>
<td>NA</td>
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<td>13</td>
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<td>220</td>
<td>127</td>
<td>66</td>
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NA = Not Analyzed; δ¹⁸O = Rsample/Rstandard-1, where R = ₁₈O/₁₆O; δ¹⁵N = Rsample/Rstandard-1, where R = ₁⁵N/₁⁴N; δ³⁷Cl = Rsample/Rstandard-1, where R = ³⁷Cl/³⁵Cl; Δ¹⁷O = [(1+Δ¹⁷O)/(1+Δ¹⁸O)⁰.⁵²⁵]-1; ‰ = parts per thousand; δ³⁷Cl, δ¹⁸O, and Δ¹⁷O values are referenced to 0 for SMOC, VSMOW, and VSMOW, respectively.
Table 2.1.4  Selected chemical and isotopic data for groundwater samples from the southwestern United States and Chile.

<table>
<thead>
<tr>
<th>Well</th>
<th>Location</th>
<th>ClO₄⁻</th>
<th>NO₃⁻-N</th>
<th>Cl⁻</th>
<th>O₂</th>
<th>³H</th>
<th>Dissolved Inorganic Carbon ¹⁴C</th>
<th>ClO₄⁻</th>
<th>ClO₄⁻</th>
<th>ClO₄⁻</th>
<th>NO₃⁻</th>
<th>NO₃⁻</th>
<th>NO₃⁻</th>
<th>δ¹⁸O</th>
<th>δ¹⁸O</th>
<th>Δ¹⁷O</th>
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<tr>
<td>MW2A</td>
<td>SHP, Texas and New Mexico, USA</td>
<td>24</td>
<td>2.9</td>
<td>1,300</td>
<td>6.8</td>
<td>2.5 (0.2)</td>
<td>38.4 (0.3)</td>
<td>+4.2</td>
<td>+1.4</td>
<td>+0.2</td>
<td>+5.5</td>
<td>+2.0</td>
<td>+0.3</td>
<td>+3.1</td>
<td>+2.2</td>
<td>+0.3</td>
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<td>MW2B</td>
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<tr>
<td>MW3A</td>
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<td>19</td>
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<td>+2.2</td>
<td>+0.3</td>
<td>+6.6</td>
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<tr>
<td>GW2</td>
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<td>98.1 (0.4)</td>
<td>+5.0</td>
<td>+3.8</td>
<td>+0.2</td>
<td>+19.5</td>
<td>+16.0</td>
<td>NA</td>
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<tr>
<td>BW2</td>
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<td>1.8</td>
<td>1.7</td>
<td>61</td>
<td>7.6</td>
<td>0.1 (0.2)</td>
<td>83.5 (0.5)</td>
<td>+4.5</td>
<td>+0.55</td>
<td>+0.6</td>
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<td>+3.7</td>
<td>NA</td>
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<td>99.7 (0.4)</td>
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<td>+0.5</td>
<td>+9.9</td>
<td>+5.2</td>
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<td>430</td>
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<td>+4.8</td>
<td>+0.8</td>
<td>+6.3</td>
<td>+0.3</td>
<td>+0.1</td>
<td></td>
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</tr>
<tr>
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<td>11.6 (0.2)</td>
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<td>+1.5</td>
<td>+1.2</td>
<td>+4.8</td>
<td>+1.0</td>
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<td>5.9 (0.1)</td>
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<td>+1.9</td>
<td>+1.3</td>
<td>+4.9</td>
<td>-1.0</td>
<td>NA</td>
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<tr>
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<td>NA</td>
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<td>+49.9</td>
<td>+16.5</td>
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</table>

aNA = Not Analyzed; bδ¹⁸O = Rsample/Rstandard-1, where R= ¹⁸O/¹⁶O; cδ¹⁵N = Rsample/Rstandard-1, where R= ¹⁵N/¹⁴N; dδ³⁷Cl = Rsample/Rstandard-1, where R= ³⁷Cl/³⁵Cl; eTU = Tritium Units = 10¹⁸ x ³H atoms/H atoms. fpmc= percent modern carbon (not normalized for δ¹³C); gΔ¹⁷O= [(1+δ¹⁷O)/(1+δ¹⁸O)⁰.⁵²⁵]-1; h‰ = parts per thousand; δ³⁷Cl, δ¹⁸O, and Δ¹⁷O values are referenced to 0 for SMOC, VSMOW, and VSMOW, respectively

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Figure 2.1.2. Summary of new isotope data for ClO₄⁻ from Southern High Plains (SHP) and Middle Rio Grande Basin (MRGB) groundwater, SHP unsaturated sub-soil, Death Valley and Atacama caliches displayed with previously published ClO₄⁻ isotope data (Böhlke et al., 2005; Sturchio et al., 2006; Bao and Gu, 2004). Arrows represent microbial fractionation slopes (Sturchio et al., 2007; Hatzinger et al., 2009), direction of mass dependent fractionation, direction of ³⁶Cl decay, and direction due to oxygen exchange. δ³⁷Cl, δ¹⁸O, and Δ¹⁷O values are referenced to 0 for SMOC, VSMOW, and VSMOW, respectively. ³⁶Cl values are from Sturchio et al., (2009) with the exception of the Zabriskie sample (Death Valley caliche with the highest Δ¹⁷O) (Table 2.1.3). δ¹⁸O = R_{sample}/R_{standard}-1, where R=¹⁸O/¹⁶O; δ³⁷Cl = R_{sample}/R_{standard}-1, where R=³⁷Cl/³⁵Cl and Δ¹⁷O= [(1+δ¹⁷O)/(1+ δ¹⁸O)]⁰.⁵²⁵-1.
Figure 2.1.3. Comparison of NO$_3^-$/ClO$_4^-$ and Cl$^-$/ClO$_4^-$ molar ratios in new samples with previously published values for wet deposition across the contiguous United States (excluding coastal sites) (Rajagopalan et al., 2009) and vadose-zone salt accumulations in the southwestern United States (Rao et al., 2007). The reported average Cl$^-$/ClO$_4^-$ ratio is shown for groundwater (GW) in the SHP (Rajagopalan et al., 2006).
Figure 2.1.4. Relations between $\Delta^{17}O$ and $\delta^{18}O$ in ClO$_4^-$ and NO$_3^-$ (data from this study and Böhlke et al., 2005; Sturchio et al., 2006; Bao and Gu, 2004; USGA, 2010; Rao et al., 2010). $\delta^{18}O$ and $\Delta^{17}O$ values are referenced to 0 for VSMOW (Böhlke et al., 2005). A hypothetical trend line is shown for NO$_3^-$ mixtures consisting of biogenic and atmospheric (O$_3$-generated) endmembers. A hypothetical trend line through Atacama and Death Valley ClO$_4^-$ could indicate varying expression of O$_3$-generated components.
Figure 2.1.5. Distribution of ClO$_4^-$ and other anions with depth below land surface at a rangeland site in the SHP, western Texas. The water table was more than 15 m below land surface. Concentrations are given as mass of leachable anions per mass of solid material that was leached. Sample SHP-V was taken from 200-400 cm below land surface at this site.
2.1.4 Isotopic Constraints on Origins of Natural Perchlorate

The data obtained in this task expand the range of known isotopic variation in natural ClO₄⁻, based on a combination of δ³⁷Cl, δ¹⁸O, and Δ¹⁷O data. Three major sample groups compared in this study have distinctive isotopic characteristics: Atacama caliche-type ClO₄⁻, Death Valley caliche-type ClO₄⁻, and SHP unsaturated-zone and groundwater ClO₄⁻ (Figure 2.1.2). The most variable of these individual groups is the Death Valley samples, which exhibit a wide range and positive correlation of δ¹⁸O and Δ¹⁷O values, including the highest values reported to date for ClO₄⁻ (Figure 2.1.4). Isotopic differences within and among these groups could be variably related to: (1) isotopic compositions of precursor compounds prior to ClO₄⁻ formation, (2) isotopic fractionations accompanying ClO₄⁻ formation, (3) isotopic exchange between ClO₄⁻ and associated chemical species such as H₂O in the environment, and (4) kinetic isotopic fractionation caused by ClO₄⁻-consuming reactions such as microbial reduction. The importance of these different effects and their relation to regional variation in terrestrial ClO₄⁻ isotopic composition are not completely resolved. Additional constraints are provided by laboratory experiments, ³⁶Cl concentrations in ClO₄⁻, and O isotopes in NO₃⁻.

One possible explanation for isotopic differences between different natural ClO₄⁻ occurrences is different mechanisms and (or) locations of ClO₄⁻ formation. High Δ¹⁷O values presumably indicate O transfer from O₃ during photochemical Cl oxidation in the atmosphere, whereas low Δ¹⁷O values could indicate photochemical processes involving oxidants other than O₃, possibly occurring in the atmosphere or at the Earth’s surface (see Section 2.1). Experiments indicate that ClO₄⁻ can be produced from reactions of oxy-chlorine intermediates (HOCl, ClO₂⁻) by irradiation with UV or sunlight (Section 2.1; Kang et al., 2006; Rao et al., 2010). Chlorine dioxide (ClO₂) exists in the stratosphere and may be a precursor compound for a non-O₃-mediated process of ClO₄⁻ generation (Solomon et al., 1989). The apparent correlation between Δ¹⁷O and δ¹⁸O values in both Atacama and Death Valley ClO₄⁻ could be interpreted as a mixing line between O₃ mediated and non-O₃-mediated production mechanisms similar to atmospheric NO₃⁻ (Figure 2.1.4). However, this hypothesis may be difficult to reconcile with the geographic distributions of ³⁶Cl/Cl ratios and Δ¹⁷O values in ClO₄⁻. Data for a subset of our samples indicate relatively high concentrations of cosmogenic ³⁶Cl in natural ClO₄⁻ from both the Death Valley and SHP occurrences, and lower concentrations in Atacama ClO₄⁻ (Figure 2.1.2; Sturchio et al., 2009). High ³⁶Cl/Cl ratios in the U.S. samples (8,000-28,000 × 10⁻¹⁵) seem to preclude formation of ClO₄⁻ from common Cl precursors in the troposphere or near the land surface. The ³⁶Cl data could be consistent with ClO₄⁻ having formed in the upper atmosphere and deposited onto the land surface, followed by varying amounts of radioactive decay depending on the accumulation times of the different deposits (of the order of 10⁶-10⁷ years in the Atacama and 10⁴ years in Death Valley and the SHP) (Sturchio et al., 2009). If the bulk of the ClO₄⁻ in all of these occurrences formed in the stratosphere and was unreactive after deposition, then differences in the measured stable Cl and O isotopes would seem to require spatial variations in either the formation mechanisms or the isotopic compositions of precursor compounds in the atmosphere.
However, the large range of $\Delta^{17}$O values locally among the Death Valley samples, and the near absence of elevated $\Delta^{17}$O values in the other southwestern U.S. ClO$_4^-$ samples compared to the Death Valley samples, would be difficult to rationalize on the basis of high-altitude source variations.

Alternatively, post-deposition alteration of ClO$_4^-$ isotopic composition could account for local variations in the stable isotopic composition of atmospherically produced ClO$_4^-$, and it could be related to similar processes affecting the isotopic composition of atmospheric NO$_3^-$. Microbial reduction of ClO$_4^-$ is known to occur in soils and groundwaters under suboxic conditions, and it is known to cause large fractionation effects in Cl and O isotopes (Sturchio et al., 2007; Hatzinger et al., 2009). However, available data indicate these isotope fractionation effects would not be consistent with many of the differences observed among the natural ClO$_4^-$ sample groups in Figure 2.1.2 (e.g., microbial reduction follows a specific trajectory in $\delta^{37}$Cl and $\delta^{18}$O and would not alter $\Delta^{17}$O substantially because it is mass-dependent), so biodegradation is not considered to be the major cause of the observed differences.

Our data indicate strong positive correlations between $\Delta^{17}$O and $\delta^{18}$O values in ClO$_4^-$ and NO$_3^-$ from Death Valley and the Atacama Desert (Figure 2.1.4). Both compounds had relatively high $\Delta^{17}$O in hyper-arid and barren settings (Atacama and Death Valley clay hills), although the relative positions of Atacama and Death Valley samples were reversed for the two compounds. Both compounds had relatively low $\Delta^{17}$O values in less arid settings. For NO$_3^-$, although atmospheric production mechanisms have been proposed to vary both spatially and temporally, published analyses indicate consistently high mean annual $\Delta^{17}$O values in atmospheric NO$_3^-$ from many regions of the world, and it is considered likely that the full range of $\Delta^{17}$O in terrestrial NO$_3^-$ is largely a reflection of varying proportions of atmospheric NO$_3^-$ (with high $\Delta^{17}$O) and biogenic NO$_3^-$ (with $\Delta^{17}$O at or near 0) (Michalski et al., 2003, 2004; Ewing et al., 2007). In the current study, the highest $\Delta^{17}$O values in NO$_3^-$ (+17 to +21 ‰) were from the Atacama Desert and could indicate 60-80 % of the NO$_3^-$ was unaltered atmospheric NO$_3^-$, assuming a long-term average atmospheric NO$_3^-$ $\Delta^{17}$O value of +25 ‰ (McMahon et al., 2006). Somewhat lower $\Delta^{17}$O values in NO$_3^-$ from the Death Valley region (+7 to +15 ‰) could indicate somewhat lower fractions of atmospheric NO$_3^-$ (30-60 %), based on the same assumption. Much lower NO$_3^- \Delta^{17}$O values were obtained from two of the SHP samples (+0.1 and +0.3 ‰), indicating almost no unaltered atmospheric NO$_3^-$.

From the strong correlation between values of $\Delta^{17}$O and $\delta^{18}$O for NO$_3^-$ (Figure 2.1.4), we infer that the generally low $\delta^{18}$O values of the other groundwater samples from SHP and MRGB also indicate little or no unaltered atmospheric NO$_3^-$ in those samples. The presence or absence of atmospheric isotopic characteristics in NO$_3^-$ in these environments is qualitatively consistent with the potential for biological N cycling in local soils (assimilation, N$_2$ fixation, mineralization, and nitrification) (Figure 2.1.6). These processes are expected to be more active in the SHP than in the Atacama Desert or the clay hills of Death Valley because of the low precipitation and general absence of plant life and organic soils in the latter environments. However, whereas nitrification of reduced
N (including atmospheric NO$_3^-$ formerly assimilated into biota and re-mineralized) is a well-documented mechanism for diluting or replacing the atmospheric $\Delta^{17}$O signature of deposited NO$_3^-$, a comparable mechanism for similarly altering the isotopic composition of ClO$_4^-$ has not been shown. Therefore, although terrestrial biologic processes can account for O isotopic variations in NO$_3^-$, and although the distribution of $\Delta^{17}$O variations in both NO$_3^-$ and ClO$_4^-$ appear to be related spatially to local biologic activity, it is not yet possible to attribute ClO$_4^-$ isotopic variation to the same processes that generally are thought to affect NO$_3^-$ isotopes in these environments.

Another possible explanation for local variations in natural terrestrial ClO$_4^-$ derived from the atmosphere is post-deposition isotope exchange (partial equilibration). Isotope exchange between ClO$_4^-$ and H$_2$O would be expected to cause a decrease in $\Delta^{17}$O and could be consistent with the ClO$_4^-$ isotope data if exchange was more advanced in wetter or more biologically active environments. Equilibrium O isotope fractionation factors between ClO$_4^-$ and H$_2$O are not known, but we expect $\delta^{18}$O of ClO$_4^-$ could be higher than $\delta^{18}$O of coexisting H$_2$O by analogy with reported fractionation effects ranging from about +14 to +30 ‰ for SO$_4^{2-}$, HSO$_4^-$, NO$_3^-$, and NO$_2^-$ at room temperature (Mizutani et al., 1969; Zeebe et al., 2010; Böhlke et al., 2003; Casciotti et al., 2007). Therefore, both the $\Delta^{17}$O and $\delta^{18}$O values of SHP and MRGB ClO$_4^-$ could be qualitatively consistent with partial O isotopic exchange of Death Valley-type ClO$_4^-$ with local H$_2$O ($\delta^{18}$O = -13 to -5 ‰) (Figure 2.1.4). Theoretical calculations indicate that $\delta^{37}$Cl of ClO$_4^-$ could be 73 ‰ higher than that of coexisting Cl$^-$ at equilibrium (Schauble et al., 2003). Thus, the relatively high $\delta^{37}$Cl values of SHP and MRGB ClO$_4^-$ might indicate partial exchange of Cl isotopes between ClO$_4^-$ and Cl$^-$ (or another Cl species) with increasing moisture and(or) biologic activity if the ClO$_4^-$ source(s) had relatively low $\delta^{37}$Cl, as in Atacama or Death Valley ClO$_4^-$. However, it is difficult to envision a mechanism that could accomplish Cl isotope exchange, especially in the absence of O isotope exchange (e.g., to explain differences between Atacama and Death Valley $\delta^{37}$Cl and $\Delta^{17}$O). Limited data indicate abiotic exchange of O isotopes between ClO$_4^-$ and H$_2$O is slow, if it occurs at all, in simple laboratory experiments [time constant > 100 years, Hoering et al. (1958); > 4500 years, Hatzinger et al. (2011)], but exchange might be catalyzed by other solid or aqueous species in soils and groundwaters, perhaps including organic compounds, as demonstrated for NO$_3^-$ and PO$_4^{3-}$ (Böhlke et al., 2003; Blake et al., 1997; Bunton et al., 1953). Field data indicate that Atacama ClO$_4^-$ introduced into humid soils and groundwaters in the eastern U.S., and synthetic ClO$_4^-$ contamination in groundwater in southern Nevada, did not exchange Cl or O isotopes substantially, despite groundwater residence times of the order of 30-40 years (Böhlke et al., 2005, 2009), although potential catalyzed exchange in unsaturated soils may have been precluded in those particular settings by rapid infiltration and recharge. Thus, although post-depositional isotope exchange could provide an explanation for some of the local natural ClO$_4^-$ isotopic variations, it is not possible to predict rates of exchange with certainty, and it is not clear if any simple exchange model would produce the range of natural ClO$_4^-$ isotopic compositions observed.
2.1.5 Implications for Perchlorate Isotope Forensics

Given present information, it is not yet possible to fully explain the observed variations in the isotopic composition of natural ClO₄⁻ sources, other than to say that some of the Atacama and Death Valley ClO₄⁻ probably formed as a result of reactions with O₃. The data permit the interpretation that natural ClO₄⁻ may have more than one formation mechanism, there may be global variations in the isotopic compositions of precursor compounds, and it may be subject to isotopic modification in the terrestrial environment. Resolving these issues would contribute to understanding atmospheric Cl chemistry, as well as supporting the basis of the isotopic approach for quantifying ClO₄⁻ sources in the environment. Nevertheless, despite uncertainty about processes responsible for some of the isotopic variations, this study indicates that natural ClO₄⁻ indigenous to the southwestern U.S. is distinguishable from synthetic ClO₄⁻ and from imported Atacama ClO₄⁻ on the basis of isotopic composition. These differences in isotopic composition may find important applications in resolving questions of ClO₄⁻ source apportionment for contaminated water supplies.

![Graph](image)

**Figure 2.1.6. Relation between δ¹⁵N and δ¹⁸O of NO₃⁻ in groundwater and leachate samples.** A rough inverse correlation between δ¹⁵N and δ¹⁸O shown here is consistent with varying mixtures of natural biogenic NO₃⁻ and atmospheric NO₃⁻ indicated by a positive correlation between δ¹⁷O and δ¹⁸O (Figure 2.1.4).
2.2 Occurrence and Stable Isotope Analysis of Perchlorate in the U.S. Great Lakes

2.2.1 Background

Initial measurements conducted during this research project demonstrated that ClO₄⁻ is present at low concentration throughout the water of the Great Lakes. The only previously published report of ClO₄⁻ concentrations in the Great Lakes was for surface water samples from Lakes Huron and Erie, and Hamilton Harbour of Lake Ontario, by Backus et al. (2005). However, only a few samples from Hamilton Harbour and some Canadian river water samples had detectable ClO₄⁻ in the range of 0.16 to 0.33 µg/L, and concentrations in all samples from the open waters of the Great Lakes were below that study’s method detection limit of 0.19 µg/liter (Backus et al., 2005).

The Great Lakes formed about 10,000 to 15,000 years ago in basins excavated by kilometers-thick glaciers (Hough, 1963; Larson and Schaeztl, 2001). The five-lake system contains about 20 percent of the world’s surface freshwater and 95 percent of the surface freshwater of the US, thus making this lake system one of the world’s most important and valuable natural resources. The Great Lakes have vital importance for drinking water, and other industrial and agricultural water needs in the region. Over thirty million people in the US and in Canada depend on the Great Lakes for their drinking water supply (Rheaume et al., 1995; Pearson et al., 2010; EPA, 2000).

We hypothesize that most of the ClO₄⁻ present in the Great Lakes is from direct atmospheric deposition, and thus its isotopic composition may be representative of the indigenous atmospheric ClO₄⁻ in the region with possible modification by processes occurring in the water column and/or the watershed (Poghosyan et al., 2014). The objective of this task was to quantify ClO₄⁻ inventories in the Great Lakes, characterize isotopic compositions of ClO₄⁻ (Δ¹⁷O, δ¹⁸O, δ³⁷Cl, ³⁶Cl/Cl), and to use these measurements to identify the source(s) of ClO₄⁻, elucidate its general geochemical behavior, and evaluate the potential extent of its biodegradation in the Great Lakes.

2.2.2 Materials and Methods

2.2.2.1 Sample Collection

Great Lakes water samples were collected aboard the US Environmental Protection Agency’s R.V. Peter Wise Lake Guardian during two routine monitoring cruises in August 2007 and August 2008. Samples for concentration analysis were collected from Lakes Michigan and Huron in August 2007, and we tested a method using multiple 100-mL ion-exchange resin columns to concentrate perchlorate from lake water for isotopic analysis. A more complete set of samples from all five lakes was collected for concentration analysis, and isotopic samples were collected on 1-liter resin columns in August 2008. The ship departed Milwaukee, WI on August 1, 2008, and sampled the lakes in the following sequence: Michigan, Huron, Erie, Ontario, and Superior, reaching Duluth, MN on August 24, 2008. A total of 74 unfiltered water
samples (~100 mL each) for ClO₄⁻ concentration analysis were collected from a rosette sampler at EPA monitoring stations, including at least one depth profile for each lake (Morrison, 2009).

Perchlorate samples for isotopic analysis were extracted from large amounts of lake water by using one-liter clear PVC columns filled with Purolite A530E bifunctional anion exchange resin. Similar columns with a 100-mL volume have been used extensively to collect perchlorate from groundwater (e.g., Section 2.2; Böhlke et al., 2009). Water was pumped up from a port at one-meter depth to an on-board laboratory and through two sediment filters in series (1st coarse + 2nd fine) to remove particles down to about 5 µm nominal size. A second pump was placed after the filters to push water through the 1 L ion exchange resin column. Water was passed continuously through a single resin column at a rate of ~10 L/min for the duration of the traverse on each lake. A new column was used for each lake. The total water volumes passed through the resin columns were: Lake Superior (20,430 L), Lake Michigan (31,780 L), Lake Huron (23,780 L), Lake Erie (15,260 L), and Lake Ontario (19,700 L). The sediment filters were changed two or three times per lake. Residence time in the two filter housings was not more than about three seconds each, and the water being passed through the filters was oxic, therefore the likelihood of any ClO₄⁻ biodegradation as water passed through the filter housings was minimal. Concentration samples were also collected before and after the resin columns to determine the efficiency of ClO₄⁻ extraction from water. All samples were stored in a walk-in freezer aboard the ship, and transferred from the ship to the Environmental Isotope Geochemistry Laboratory of the University of Illinois at Chicago in coolers with ice, where they were refrigerated until analysis.

The amount of ClO₄⁻ recovered from the Lake Superior sample from August 2008 was not sufficient for the planned isotopic analyses, so a second sampling trip was conducted to Lake Superior during September 24 - 28, 2010 to collect additional samples at the water filtration plant of Marquette, MI. Marquette was chosen because no major rivers discharge to Lake Superior in the region. The plant produces about 3 million gallons of Lake Superior water per day from an intake located 400 m off shore at about 18.3 m depth. Two one-liter sample columns were collected at Marquette, one from the raw intake water and another from the pre-filtered (500 µm) water supply. Fiberglass filters and glass wool fillings inside the perchlorate columns became clogged with sediments during sample collection, and these were changed daily to maintain flow through the columns. Total volumes of 86,000 L (Superior R) and 73,000 L (Superior P) were passed through raw and pre-filtered water-sample resin columns, respectively.

2.2.2.2 Concentration Analysis
Perchlorate concentrations in all of our water samples were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) at the Environmental Analysis Laboratory of Texas Tech University as previously described in Section 2.1, 2.2 and elsewhere (Rajagopalan et al., 2006, Rao et al., 2007). All samples were spiked with an oxygen isotope (¹⁸O) labeled ClO₄⁻ internal standard. Sample concentrations were determined from the ratio of the sample
ClO₄⁻ peak to the internal standard peak, with a reporting limit of 0.05 μg/L based on the lowest calibration point. The method detection limit and limit of quantification have previously been defined as 7 ng/L and 18 ng/L, respectively (Rajagopalan et al., 2009, Rao et al., 2012, Rao, 2010). The samples were analyzed in batches of eight followed by an analytical duplicate, spike, blank and a continuing calibration check (CCC). The tolerance for deviation in the duplicate samples and CCC was 20%, and for recovery of sample spike was 80 to 120%. If any one of the above conditions was not met, or if ClO₄⁻ in the laboratory reagent blank was greater than 0.3 times the quantification limit, then the results of that batch were discarded and the samples were reanalyzed.

2.2.2.3 Perchlorate Elution for Isotopic Analysis.

The 1L resin columns containing adsorbed ClO₄⁻ were first flushed with 4 bed volumes of 4 mol/L HCl in order to elute anions other than perchlorate and 2 bed volumes of ethanol to remove organic material coating the resin. The columns were then flushed with 1 bed volume of deionized water and the resin was removed for ultrasonic cleaning in deionized water. After these steps, ClO₄⁻ was eluted by tetrachloroferrate (FeCl₄⁻) in a solution containing 1 mol/L FeCl₃ and 4 mol/L HCl (Section 2.2; Bao and Gu., 2004; Gu et al., 2001; 2007). The ClO₄⁻-bearing eluent was diluted with deionized water (to convert tetrachloroferrate to cationic Fe species) and Fe was removed by passing the solution through a column of Bio-Rad AG50W-X12 cation exchange resin. The solution was then evaporated to a volume of about 25 mL following addition of a few milliliters of 30 % H₂O₂. An aliquot of this solution was used for ion chromatographic analysis of the amount of ClO₄⁻ recovered from the sample column. The solution was then neutralized to a pH of just above 1 using 10 mol/L NaOH, and reloaded onto 2 mL of resin for a repeat of the elution and Fe-removal steps described above. The eluent solution was then evaporated down to ~0.5 mL to remove HCl. The solution was then diluted with 3 mL deionized water and pushed through Ag-treated On-Guard columns for Cl⁻ removal and Ba-treated cation-exchange columns for SO₄²⁻ removal. The solution was then passed through solid-phase extraction (SPE) columns for removal of organics, and finally mixed with excess CsOH to precipitate CsClO₄. Final purification of perchlorate samples were conducted by precipitation and recrystallization of tetra-n-pentylammonium perchlorate (TPAClO₄) salts (Dosch, 1968). The sample processing blank for perchlorate was about 50 μg (from reagents), comprising <5 % of the perchlorate recovered for isotopic analysis.

2.2.2.4 Isotopic Analysis of Perchlorate by SIMS

Stable isotope ratios of O and Cl in perchlorate are normally analyzed by isotope-ratio mass spectrometry (IRMS) as detailed in Section 2.2. However, we used an alternative method to maximize the amount of data we could obtain from the small amounts of perchlorate recovered from the Great Lakes. Secondary ion mass spectrometry (SIMS) measurements of O and Cl isotope ratios were performed on TPAClO₄. Samples were mounted by pressing into indium metal in a 2.5-cm diameter sample holder and coating the surface with 50 nm of gold. The samples were analyzed at the Center for Microanalysis of California Institute of Technology by
using the CAMECA IMS 7f-GEO SIMS instrument. Analyses were performed with a 10 keV Cs⁺ primary ion beam. The ion beam was focused to a diameter of ~20 µm with 5-6 nA of beam current. All isotope ratio analyses were performed by pre-sputtering of a 100 × 100 µm raster area for 120 sec, and then scanning a 75 × 75 µm raster during data collection. ³⁴SΔ contribution to ³⁵Cl results was negligible at a mass resolving power (MRP) of 1200; this was also confirmed under MRP of 5000. A higher MRP of 6500 was used for oxygen isotope ratio measurements, but it was sometimes difficult to eliminate the effect of large ¹⁶OH interference on ¹⁷O. This ¹⁶OH interference caused relatively large errors for the ¹⁷O results, therefore SIMS results for ¹⁷O/¹⁶O ratios are not reported. An energy bandwidth of 45 eV was set for all SIMS measurements of both chlorine and oxygen isotopes. Secondary ion accelerating voltage was -9 keV for all isotopic analyses. For oxygen isotopes, a Faraday cup was used for ¹⁶O, and an electron multiplier for ¹⁷O and ¹⁸O, with data collection times of 1 sec, 10 sec, and 2 sec per cycle, respectively, for a total of 100 cycles. Chlorine isotopes ³⁵Cl and ³⁷Cl were measured by Faraday cups with data collection times of 1 sec and 2 sec per cycle, respectively, for a total of 20 cycles. Data are normalized to measurements of KClO₄ isotopic reference materials USGS37 and USGS38, which were converted to TPAClO₄. Stable isotope ratios are reported in the conventional delta (δ) notation, as follows:

\[
\delta^{18}O (\text{‰}) = \left[\frac{(^{18}O/^{16}O)_{\text{sample}}}{(^{18}O/^{16}O)_{\text{VSMOW}}} - 1\right] \times 1000
\]

\[
\delta^{37}Cl (\text{‰}) = \left[\frac{(^{37}Cl/^{35}Cl)_{\text{sample}}}{(^{37}Cl/^{35}Cl)_{\text{SMOC}}} - 1\right] \times 1000
\]

where VSMOW and SMOC are the isotopic references Vienna Standard Mean Ocean Water (Coplen, 1996) and Standard Mean Ocean Chloride (Godon et al., 2004), respectively. Analytical precisions (1σ) based on replicate measurements of reference materials are ±1.8 ‰ for δ¹⁸O and ±1.0 ‰ for δ³⁷Cl. SIMS produced acceptable results for δ¹⁸O and δ³⁷Cl analysis but ¹⁷O/¹⁶O measurements encountered difficulties in eliminating the effect of large ¹⁶OH interference on ¹⁷O (which may have been related to the use of TPAClO₄ as a target).

2.2.2.5. Isotopic Analysis of Perchlorate by Dual-inlet IRMS

Because of the difficulty encountered in precise determination of ¹⁷O/¹⁶O ratios by SIMS, samples were converted from TPAClO₄ to KClO₄ by using 0.5 mol/L KOH in absolute ethanol solution, for measurement of oxygen isotope ratios by dual-inlet IRMS of O₂ produced by sealed-tube decomposition of KCO₄ at 600°C. Reported ¹⁷O/¹⁶O ratios are given in units of Δ¹⁷O, where:

\[
\Delta^{17}O (\text{‰}) = [(1 + \delta^{17}O/1000) / (1 + \delta^{18}O/1000)^{0.525}] - 1] \times 1000
\]

Analytical precision of Δ¹⁷O based on replicate measurements of USGS-37 KClO₄ isotopic reference material is ±0.2 ‰.
2.2.2.6 $^{36}$Cl Abundance Analysis by AMS

Accelerator mass spectrometry (AMS) measurements of $^{36}$Cl isotopic abundances in AgCl (prepared from the KCl produced by decomposition of KClO$_4$ for IRMS $\Delta^{17}$O analyses) were conducted at the Purdue Rare Isotope Measurement (PRIME) Laboratory at Purdue University. Prior to precipitation of AgCl, the chloride was purified by the standard ion chromatography developed by the PRIME Lab for $^{36}$Cl analyses. The $^{36}$Cl results were corrected for the instrumental background and are reported in units of $^{36}$Cl/Cl × 10$^{-15}$ along with errors based on counting statistics.

2.2.3 Results and Discussion

2.2.3.1 Perchlorate Concentrations

A group of 74 samples were analyzed for ClO$_4^-$ concentrations in the Great Lakes, and the concentrations ranged from 0.05 to 0.13 µg/L (averages per lake are provided in Table 2.2.1 and all data are provided in Table 2.2.2). The profiles of ClO$_4^-$ concentrations with depth in each lake are graphed in Figure 2.2.1. Although the perchlorate concentrations in some of the samples are just above the reporting limit, they all exceed the method detection limit by a factor of seven or more. Depth profiles of ClO$_4^-$ concentration were determined for two locations on Lake Superior and one location on each of the other lakes (Figure 2.2.1). Concentrations were nearly constant with depth. The lack of stratification reflects vertical mixing caused by seasonal overturns of the water column (Fuller et al., 1995). Therefore, we consider our data to be fairly representative of the concentrations and isotopic compositions of perchlorate in each of the lakes. All water samples collected after the ion-exchange resin columns had perchlorate concentrations below the reporting limit of 0.05 µg/L, consistent with perchlorate removal by the resin columns.
Table 2.2.1. Concentrations and isotopic compositions of perchlorate from the Great Lakes.

<table>
<thead>
<tr>
<th>Lakes</th>
<th>Sample volume (L)</th>
<th>Mean ClO₄⁻ (µg/L)</th>
<th>δ³⁷Cl (‰)</th>
<th>δ¹⁸O (‰)</th>
<th>Δ¹⁷O (‰)</th>
<th>⁴⁰Cl/Cl (10⁻¹⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior Rᵇ</td>
<td>86000</td>
<td>0.06</td>
<td>+3.9</td>
<td>-4.8</td>
<td>+2.7</td>
<td>71200 ± 1700</td>
</tr>
<tr>
<td>Superior Pᵇ</td>
<td>73000</td>
<td>0.06</td>
<td>+4.0</td>
<td>-3.4</td>
<td>+2.6</td>
<td>61900 ± 1100</td>
</tr>
<tr>
<td>Michigan</td>
<td>31780</td>
<td>0.10</td>
<td>+3.3</td>
<td>-1.3</td>
<td>+1.7</td>
<td>26600 ± 800</td>
</tr>
<tr>
<td>Huron</td>
<td>23780</td>
<td>0.11</td>
<td>+3.2</td>
<td>-2.7</td>
<td>+1.6</td>
<td>15800 ± 600</td>
</tr>
<tr>
<td>Erie</td>
<td>15260</td>
<td>0.08</td>
<td>+3.2</td>
<td>+4.0</td>
<td>+1.8</td>
<td>9300 ± 300</td>
</tr>
<tr>
<td>Ontario</td>
<td>19700</td>
<td>0.09</td>
<td>+3.0</td>
<td>+1.9</td>
<td>+1.7</td>
<td>7400 ± 300</td>
</tr>
</tbody>
</table>

*a sample volume used for isotopic analysis. *b Superior R and Superior P are samples collected from raw and pre-filtered water supplies, respectively. *c perchlorate concentrations have standard deviation of ± 0.01 µg/L.

Figure 2.2.1. Perchlorate concentration vs. depth in the Great Lakes.
Table 2.2.2. Perchlorate concentrations in the water samples from the Great Lakes.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Date</th>
<th>Station</th>
<th>Depth (m)</th>
<th>ClO$_4^-$ (μg/L)</th>
<th>Mean ClO$_4^-$ (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior</td>
<td>August, 2008</td>
<td>SU01M</td>
<td>1.5</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August, 2008</td>
<td>SU01M</td>
<td>5.1</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August, 2008</td>
<td>SU01M</td>
<td>10.5</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August, 2008</td>
<td>SU01M</td>
<td>13.1</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August, 2008</td>
<td>SU01M</td>
<td>18.8</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August, 2008</td>
<td>SU01M</td>
<td>33</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August, 2008</td>
<td>SU01M</td>
<td>40.2</td>
<td>0.06</td>
<td></td>
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<td>Sea Chest</td>
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August, 2007  |  HU53 SURF  |  Surface  |  0.10  
August, 2007  |  HU61 SURF  |  Surface  |  0.10  
August, 2008  |  HU15M  |  Sea Chest  |  0.12  
August, 2008  |  HU45M  |  Sea Chest  |  0.12  |  0.11 ± 0.01  

Erie  
August, 2008  |  ER78M  |  0  |  0.10  
August, 2008  |  ER78M  |  2.5  |  0.08  
August, 2008  |  ER78M  |  10.4  |  0.08  
August, 2008  |  ER78M  |  11.4  |  0.08  
August, 2008  |  ER78M  |  14  |  0.08  
August, 2008  |  ER78M  |  16.4  |  0.08  
August, 2008  |  ER78M  |  21.4  |  0.08  |  0.08 ± 0.01  

Ontario  
August, 2008  |  ON33M  |  1.5  |  0.09  
August, 2008  |  ON33M  |  5  |  0.09  
August, 2008  |  ON33M  |  12.7  |  0.09  
August, 2008  |  ON33M  |  40.1  |  0.09  
August, 2008  |  ON33M  |  50.1  |  0.09  
August, 2008  |  ON33M  |  99.9  |  0.09  
August, 2008  |  ON33M  |  127  |  0.10  
August, 2008  |  ON33M  |  135.8  |  0.09  |  0.09 ± 0.01  

\(^{a}\) The “sea chest” is a port through the ship’s hull about one meter below water level. Water was pumped continuously through this port up to the laboratory for sampling of perchlorate on 1-liter ion-exchange resin columns for isotopic analysis.

2.2.3.2 Perchlorate Isotopic Compositions
The isotopic compositions of ClO\(_4^-\) extracted from the Great Lakes provide constraints on possible source(s) and may indicate geochemical processes affecting ClO\(_4^-\) concentrations in the lakes. Values of \(\delta^{37}\)Cl have a narrow range from +3.0 \(\%\) (Lake Ontario) to +4.0 \(\%\) (Lake Superior) (using the average values of the two Lake Superior samples), which is insignificant given the SIMS analytical error of ± 1.0 \(\%\). Values of \(\delta^{18}\)O have a wider range from -4.1 \(\%\) (Lake Superior) to +4.0 \(\%\) (Lake Erie) (Table 2.2.1). Mass-independent oxygen isotopic variations are evident, with \(\Delta^{17}\)O values ranging from +1.6 \(\%\) to +2.7 \(\%\). Lake Superior has a larger \(\Delta^{17}\)O value (+2.7 \(\%\)) than the other four lakes (+1.6 \(\%\) to +1.8 \(\%\)). The overall ranges of
stable isotopic compositions of Great Lakes perchlorate resemble those of indigenous natural perchlorate measured in pre-industrial groundwaters of the western USA (Section 2.2.2; Jackson et al., 2010) indicating a predominantly natural source (Figure 2.2.2). However, the observed variations in oxygen isotope ratios may also indicate mixing with other sources, or possibly reactions such as biodegradation or oxygen exchange.

Figure 2.2.2. Diagrams showing $\delta^{37}$Cl vs. $\delta^{18}$O values (upper diagram) and $\Delta^{17}$O vs. $\delta^{18}$O values (lower diagram) for Great Lakes perchlorate samples. Published isotopic data from Atacama, Death Valley, western US groundwater and unsaturated zone, and synthetic perchlorates are also displayed on the graphs (Bao and Gu, 2004; Sturchio et al., 2006; Jackson et al., 2010; Hatzinger et al., 2013). Arrow in the upper diagram shows isotopic fractionation effect during biodegradation with a slope of 0.4 (Sturchio et al., 2007). The horizontal line in the lower diagram shows the mass dependent fractionation trend. Lake Superior values are the average of Superior R and Superior P values.
The δ\(^{18}\)O values of ClO\(_4^-\) from the Great Lakes are generally divided into two groups with Lakes Superior, Michigan and Huron having more negative δ\(^{18}\)O values (-4.1 ‰ to -1.3 ‰), and Lakes Erie and Ontario having more positive δ\(^{18}\)O values (+1.9 ‰ to +4.0 ‰). The spatial variability of the δ\(^{18}\)O values of perchlorate in the Great Lakes is similar to the spatial variability of the δ\(^{18}\)O values of H\(_2\)O (-9 ‰ to -6 ‰) in the Great Lakes, and in precipitation (-15 ‰ to -7 ‰) over the Great Lakes basin (IAEA/WMO, 2013; Longstaffe et al., 2011). This indicates that the oxygen isotopic (δ\(^{18}\)O) variability of Great Lakes ClO\(_4^-\) could potentially reflect primary isotopic variability of atmospheric perchlorate in the region similar to the observed much larger range of isotopic values in Atacama and Death Valley perchlorates (Sturchio et al., 2006; Jackson et al., 2010; Section 2.2, this document).

Post depositional oxygen isotope exchange with the water in the Great Lakes could also affect ClO\(_4^-\) isotopic compositions. However, the experimentally measured rate of ClO\(_4^-\)-water oxygen isotope exchange is low; for example, a half-life of >4500 years at room temperature was found in a homogeneous perchloric acid solution by Hatzinger et al. (2011). This implies that such exchange must be catalyzed in natural settings if our results from the Great Lakes reflect the effects of oxygen exchange. Partial ClO\(_4^-\)-water oxygen exchange could thus account for the isotopic variation in perchlorate in the Great Lakes, along with other factors such as natural variability and mixing with other sources.

Chlorine-36 (\(^{36}\)Cl) is a cosmogenic radionuclide with a half-life of 301,000 years. Atmospheric \(^{36}\)Cl is primarily produced by cosmic-ray spallation of \(^{40}\)Ar (Lehmann et al., 1993; Lal et al., 1967). Isotopic abundance of \(^{36}\)Cl in Great Lakes ClO\(_4^-\) decreases from west to east, from a high \(^{36}\)Cl/Cl ratio of 6.7 \times 10^{-11} in Lake Superior to a low ratio of 7.4 \times 10^{-12} in Lake Ontario (Table 2.2.1). The \(^{36}\)Cl/Cl ratio of perchlorate in Lake Superior is higher than that reported previously for any other location (Sturchio et al., 2009; Jackson et al., 2010; Section 2.2., this document).

Great Lakes ClO\(_4^-\) generally resembles that of indigenous natural ClO\(_4^-\) from the western USA in terms of δ\(^{37}\)Cl values and \(^{36}\)Cl/Cl ratios (Figure 2.2.3). The isotopic composition of indigenous natural ClO\(_4^-\) in groundwater appears to be fairly consistent over a wide portion of North America, including West Texas, New Mexico, and Oregon (Sturchio et al., 2006, 2009; Jackson et al., 2010; Hatzinger et al., 2013; Section 2.2., this document) and similar isotopic composition is now also observed in ClO\(_4^-\) throughout the Great Lakes (Figures 2.2.2 and 2.2.3). Based on measurements performed to date, all pre-bomb indigenous natural ClO\(_4^-\) has high \(^{36}\)Cl/Cl ratios relative to synthetic and Atacama ClO\(_4^-\); the highest \(^{36}\)Cl/Cl ratios were found in indigenous ClO\(_4^-\) that may have incorporated bomb-pulse \(^{36}\)Cl. The relatively high \(^{36}\)Cl/Cl ratios of perchlorate in the lakes having longer water residence times (Lakes Superior and Michigan, 180 yr and 110 yr respectively) are consistent with retention of bomb-pulse perchlorate. Nuclear weapon tests conducted on barges in the Pacific Ocean generated large amounts of \(^{36}\)Cl by neutron activation of \(^{35}\)Cl in seawater, and injected this \(^{36}\)Cl into the stratosphere. The \(^{36}\)Cl abundance in mid-latitude precipitation increased by as much as two to three orders of magnitude during the 1952 – 1964 period (Bentley et al., 1982; Heikkila et al., 2009). This period of
elevated atmospheric $^{36}\text{Cl}$ abundance occurred within the residence times of water contained in Lakes Superior and Michigan.

![Diagram](image)

**Figure 2.2.3.** Diagram comparing $^{36}\text{Cl}/\text{Cl}$ vs. $\delta^{37}\text{Cl}$ values for perchlorate from the Great Lakes, in comparison with synthetic perchlorate, and natural perchlorate from Atacama (Chile) and the western USA. $^{36}\text{Cl}/\text{Cl}$ errors are smaller than data points. Lake Superior value is the average of Superior R and Superior P values.

### 2.2.3.3 Mass-Balance of Perchlorate in the Great Lakes

Measured $\text{ClO}_4^-$ concentrations were used to build a mass-balance model for $\text{ClO}_4^-$ in the Great Lakes. The model is similar to the mass-balance model of chloride in the Great Lakes developed by Chapra et al., (2009). The model treats the Great Lakes as a system of interconnected, well-mixed lakes. Our model assumes that the Great Lakes are in a steady state with each lake having a constant $\text{ClO}_4^-$ concentration over time, whereby the total $\text{ClO}_4^-$ mass entering each lake equals that leaving each lake. Likewise, we assume constant lake volumes because changes caused by short-term lake-level fluctuations are relatively small compared to total lake volumes. Physical parameters of the Great Lakes are summarized in Table 2.2.3 (Chapra et al., 2009; Quinn, 1982; GERL, 2012). These parameters were used in the calculations along with the average of the 1950 – 2008 monthly hydrologic data (Table 2.2.3) from the NOAA Great Lakes Environmental Research Laboratory.

Perchlorate fluxes out of the Great Lakes occur by outflow through the interconnected channels, diversion outflows, and by unknown processes that may include uptake and biodegradation.
Perchlorate input is from dry and wet atmospheric deposition (which are assumed to be equal), upstream inflow, and possibly from additional anthropogenic sources. It was assumed initially that all ClO$_4^-$ deposited on the drainage basin of the Great Lakes is transferred into the lakes within one year. Perchlorate outflows and upstream inflows were estimated from the measured average ClO$_4^-$ concentrations in the lakes, whereas ClO$_4^-$ influx from the atmospheric deposition was estimated by using a value of twice the measured average ClO$_4^-$ concentration of 14.1 ± 13.5 ng/L in rainwater (Rajagopalan et al., 2009).

Our ClO$_4^-$ mass-balance model demonstrates that atmospheric deposition could account for the majority of the ClO$_4^-$ fluxes in Lakes Superior, Michigan, Huron, and Ontario. However, some ClO$_4^-$ loss is apparent in Lake Erie, where the influx of ClO$_4^-$ is higher than its output (Table 2.2.4, Figure 2.2.4). Assuming conservatively that ClO$_4^-$ enters Lake Erie only from Lake Huron, then Lake Erie outflow contains only about 82% of the ClO$_4^-$ from its inflow, indicating that at least 18% of the ClO$_4^-$ influx in Lake Erie is removed. In a more realistic scenario, in which perchlorate influx includes the atmospheric source as defined above, the total ClO$_4^-$ removal occurring in Lake Erie would be about 26%. In addition, any unaccounted ClO$_4^-$ contribution from the anthropogenic sources would further increase the apparent ClO$_4^-$ loss in Lake Erie.
Table 2.2.3. Physical parameters of the Great Lakes. Data from Chapra et al. (2009), Quinn (1982), GLERL (2012).

<table>
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<tr>
<th>Lakes</th>
<th>Residence time (year)</th>
<th>Lake volume (km³)</th>
<th>Surface Area (km²)</th>
<th>Drainage area (km²)</th>
<th>Over Lake Evaporation (km³/year)</th>
<th>Over Lake Precipitation (km³/year)</th>
<th>Over Land Precipitation (km³/year)</th>
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<td>Superior</td>
<td>179.8</td>
<td>12,115</td>
<td>81,925</td>
<td>128,084</td>
<td>50</td>
<td>65</td>
<td>104</td>
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<tr>
<td>Michigan</td>
<td>110.2</td>
<td>4,947</td>
<td>57,291</td>
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<td>21.3</td>
<td>3,567</td>
<td>59,560</td>
<td>132,208</td>
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<td>117</td>
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<td>499</td>
<td>25,404</td>
<td>60,602</td>
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<td>24</td>
<td>56</td>
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<td>Ontario</td>
<td>7.5</td>
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<td>19,121</td>
<td>65,118</td>
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<td>17</td>
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Table 2.2.4. Perchlorate mass-balance model results. Units are tonnes/year, except total ClO₄⁻ in tonnes.

<table>
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<tr>
<th>Lakes</th>
<th>Atmospheric ClO₄⁻ Input</th>
<th>Upstream ClO₄⁻ Inflow</th>
<th>Total ClO₄⁻ Input</th>
<th>ClO₄⁻ Outflow</th>
<th>ClO₄⁻ [Input–Ouflow]</th>
<th>Total ClO₄⁻ (tonne)</th>
</tr>
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<tr>
<td>Superior</td>
<td>4.8 ± 4.6</td>
<td>n.a.</td>
<td>4.8 ± 4.6</td>
<td>4.2</td>
<td>0.6 ± 4.6</td>
<td>727</td>
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<tr>
<td>Michigan</td>
<td>4.0 ± 3.9</td>
<td>n.a.</td>
<td>4.0 ± 3.9</td>
<td>4.5</td>
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<tr>
<td>Huron</td>
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<td>8.4</td>
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<td>18.6</td>
<td>-5.5 ± 4.5</td>
<td>392</td>
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<tr>
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<td>2.3 ± 2.2</td>
<td>19.2</td>
<td>21.5 ± 2.2</td>
<td>15.8</td>
<td>5.7 ± 2.2</td>
<td>40</td>
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<tr>
<td>Ontario</td>
<td>2.2 ± 2.1</td>
<td>15.8</td>
<td>18.0 ± 2.1</td>
<td>20.7</td>
<td>-2.7 ± 2.1</td>
<td>149</td>
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The apparent ClO$_4^-$ removal in Lake Erie does not appear to cause significant isotope fractionation of oxygen and chlorine. In contrast, assuming Rayleigh-type fractionation according to the isotopic fractionation factors measured for microbial ClO$_4^-$ reduction (Sturchio et al., 2007), a 20 % ClO$_4^-$ reduction would cause about 9 ‰ increase in $\delta^{18}$O and +3.5 ‰ increase in $\delta^{37}$Cl while a 30 % reduction of ClO$_4^-$ would increase the $\delta^{18}$O and $\delta^{37}$Cl values by about +14 ‰ and +5 ‰, respectively. However, complete biodegradation of ClO$_4^-$ diffusing into the anaerobic sediments of Lake Erie (analogous to the “reactive microsites” model of Brandes and Devol (1997)) could be a plausible mechanism for ClO$_4^-$ removal from Lake Erie without changing the isotopic composition of residual ClO$_4^-$. Lake Erie has a long history of seasonal hypoxia and sediments have high oxygen demand, resulting in anoxic conditions within 2 cm of the sediment-water interface (Matisoff et al., 2005) creating conditions conducive to microbial ClO$_4^-$ reduction. Alternatively, if isotopic fractionation accompanies perchlorate removal, the ClO$_4^-$ removed and that replaced by runoff input could fortuitously have similar stable isotopic compositions.

The mass-balance model also indicates possible minor ClO$_4^-$ contributions from the drainage basins of Lakes Huron and Ontario. Perchlorate outflow exceeds inflow by at least 1.0 and 0.6 tonne/year (Table 2.2.4, Figure 2.2.4) in Lakes Huron and Ontario, respectively, indicating a possible ClO$_4^-$ contribution from other sources not accounted by the atmospheric deposition of perchlorate when assuming a value of twice the ClO$_4^-$ concentration of 14.1 ± 13.5 ng/L in rainwater. This strongly indicates that there might be a similar magnitude of ClO$_4^-$ contribution from such unaccounted sources in Lake Erie since it is close to Lakes Huron and Ontario. However, the apparent ClO$_4^-$ removal from Lake Erie is apparently much larger than the ClO$_4^-$ influx from unaccounted sources.

2.2.3.4 $^{36}$Cl Abundance of Perchlorate Formed During Atmospheric Bomb Tests.
Natural ClO$_4^-$ of recent origin has relatively high $^{36}$Cl/Cl ratios indicating an atmospheric source. Ratios of $^{36}$Cl/Cl about $8,000 \times 10^{-15}$ were measured in ClO$_4^-$ extracted from southwest US groundwater having $^{14}$C ages of more than 10 ka (Sturchio et al., 2009). Perchlorate formed in the atmosphere during the period 1952-1964 when high quantities of bomb-produced $^{36}$Cl were present could be expected to have much higher $^{36}$Cl/Cl ratios compared with ClO$_4^-$ that formed either before or after the bomb tests (Sturchio et al., 2009).

The relatively high $^{36}$ClO$_4^-$ abundances in Lakes Superior and Michigan may indicate the presence of $^{36}$Cl-enriched ClO$_4^-$ deposition during the period of elevated atmospheric $^{36}$Cl activity following thermonuclear bomb tests in the Pacific Ocean. If we assume that the $^{36}$Cl/Cl ratios of pre- and post-bomb ClO$_4^-$ was $~8,000 \times 10^{-15}$, based on measurements of pre-bomb groundwater perchlorate, then the $^{36}$Cl abundance in atmospheric perchlorate formed during the bomb tests can be estimated from our data.

The $^{36}$ClO$_4^-$ abundances in the Great Lakes may have progressively increased from that of pre-bomb conditions by addition of ClO$_4^-$ having anomalously high $^{36}$Cl/Cl ratio that formed during
1950s and early 1960s, and peaked around mid 1960s when most of the bomb-produced $^{36}\text{Cl}$ atoms had been flushed out of the atmosphere due to the relatively short stratospheric residence time of two to three years (Bentley et al., 1982; Synal et al., 1990). We assume that the production and deposition rate of $\text{ClO}_4^-$ remained constant, and only the isotopic abundance of $^{36}\text{Cl}$ changed in response to the transient bomb-pulse injection of $^{36}\text{Cl}$ into the stratosphere.

The fraction of initial water remaining in a lake at a given time can be approximated from the relationship $V_t/V = e^{-t/T}$, where $V$ is the initial volume of the water mass at $t = 0$ (lake volume), $V_t$ is the volume of the initial water mass remaining in the lake at any given time ($t$), and $T$ is the residence time of the lake (Quinn, 1992). This equation assumes that the lake is fully mixed and $\text{ClO}_4^-$ removal from the lake occurs only through the outflow. Lake Superior satisfies both criteria of being fully mixed and having $\text{ClO}_4^-$ removal almost entirely through the outflow, which might not be the case in the lower lakes. Lake Superior also has the longest residence time (~180 yr from Table 2.2.3) indicating that most of the bomb-period water (and $\text{ClO}_4^-$) is still present within the lake.

Our estimate demonstrates that about 78% of the water in Lake Superior at 2010 was also present in 1965 when the $^{36}\text{ClO}_4^-$ abundance was most likely the highest in Lake Superior. Since we assume that Lake Superior is fully mixed and $\text{ClO}_4^-$ leaves the lake only through the outflow, the water fraction is roughly equivalent to the $\text{ClO}_4^-$ fraction. Thus, Lake Superior $\text{ClO}_4^-$ at 2010 was a mixture from $\text{ClO}_4^-$ with elevated $^{36}\text{Cl}/\text{Cl}$ ratio from the 1965 peak period (78%) and post-bomb atmospheric $\text{ClO}_4^-$ with the $^{36}\text{Cl}/\text{Cl}$ ratio of about $8,000 \times 10^{-15}$ (22%). The 1965 peak $^{36}\text{Cl}/\text{Cl}$ ratio of $\text{ClO}_4^-$ in Lake Superior would have been about $83,000 \times 10^{-15}$. The estimate of the peak $^{36}\text{Cl}/\text{Cl}$ ratio at 1965 is relatively insensitive to the $^{36}\text{Cl}/\text{Cl}$ ratio of the post-bomb atmospheric $\text{ClO}_4^-$ deposition, and it only varies by less than 3% ($83,000 \pm 2,000 \times 10^{-15}$) over a range of post-bomb $^{36}\text{Cl}/\text{Cl}$ ratios varying from $1,000 \times 10^{-15}$ to $15,000 \times 10^{-15}$. Finally, this peak $^{36}\text{Cl}$ abundance of $\text{ClO}_4^-$ in Lake Superior at the end of the bomb-test period can be used to estimate the $^{36}\text{Cl}/\text{Cl}$ ratio of the total atmospheric $\text{ClO}_4^-$ deposited during the bomb test period. Lake Superior $\text{ClO}_4^-$ at 1965 was modeled as a mixture of pre-bomb (before 1952) $\text{ClO}_4^-$ with the $^{36}\text{Cl}/\text{Cl}$ ratio of about $8,000 \times 10^{-15}$ (92.5%) and $^{36}\text{Cl}$-enriched $\text{ClO}_4^-$ deposited during the thermonuclear bomb tests (7.5%). In this case, our estimate of the $^{36}\text{Cl}/\text{Cl}$ ratio of $\text{ClO}_4^-$ deposited during the thermonuclear bomb tests is about $1,000,000 \times 10^{-15}$. Sensitivity analysis demonstrates that the $^{36}\text{Cl}/\text{Cl}$ ratio of the bomb-period atmospheric $\text{ClO}_4^-$ varies by about 11% ($1,000,000 \pm 110,000 \times 10^{-15}$) for a range of $^{36}\text{Cl}/\text{Cl}$ ratios in pre-bomb atmospheric $\text{ClO}_4^-$ from $1,000 \times 10^{-15}$ to $15,000 \times 10^{-15}$. The $^{36}\text{Cl}$ abundance in natural $\text{ClO}_4^-$ during the atmospheric thermonuclear bomb tests was about two orders of magnitude higher than that of indigenous natural $\text{ClO}_4^-$ being deposited on the surface of the Earth (Sturchio et al., 2009; Jackson et al., 2010; Section 2.2, this document)
Figure 2.2.4. Estimated perchlorate fluxes in the Great Lakes. Red numbers represent total perchlorate inventories in each lake (tonne), white numbers next to the arrows represent perchlorate fluxes (tonne/year) from direct atmospheric deposition and from the drainage basins when assuming 14.1 ng/L ClO$_4^-$ concentrations in the rainwater, and orange numbers represent ClO$_4^-$ outflows from the lakes (tonne/year).
2.3 Occurrence of Natural Perchlorate and Relationship with Nitrate in Arid and Semi-Arid Environments Worldwide

2.3.1 Background

Here we present new data for natural ClO₄⁻ occurrences in selected globally distributed settings and an overview of the factors controlling ClO₄⁻ accumulation in the environment. We measured indigenous ClO₄⁻ concentrations in soil/caliche and groundwater samples from a variety of arid and semi-arid locations in Antarctica, Chile, China, southern Africa, United Arab Emirates (UAE), and the U.S. We also investigated the relationship between ClO₄⁻ and co-occurring Cl⁻ and NO₃⁻ as well as the sources and sinks of NO₃⁻ based on isotope data (δ¹⁵N, δ¹⁸O, and Δ¹⁷O). Our objectives were to evaluate the occurrence and fate of ClO₄⁻ in arid environments and the relationship of ClO₄⁻ to the better studied atmospherically deposited species NO₃⁻ and Cl⁻ as a means to understand the prevalent processes that affect the accumulation of these species over various time scales. We developed a conceptual model of ClO₄⁻ occurrence in relation to NO₃⁻ that incorporates the overall impact of biological processes to the co-occurrence of these important oxy-anions. Our results firmly establish the widespread global occurrence of ClO₄⁻, provide insights about environmental conditions controlling its distribution, and appear to indicate a new approach for evaluating arid-region biogeochemistry with particular relevance to NO₃⁻ processing and Cl⁻ cycling. Our results also may contribute to understanding the prevalence of ClO₄⁻ on Mars and its implications to the co-occurrence of NO₃⁻ and possible extraterrestrial biologic impacts on these species (Stern et al., 2015). This work significantly expands that presented in Section 2.2, which focused primarily on the southwestern US.

Soil, unsaturated subsoil, caliche-type salt deposits, and groundwater samples were collected for this study or obtained from archived samples of previous studies from sites in the U.S., Southern Africa (Namibia, South Africa, and Botswana), UAE, China, Chile, and Antarctica (Figure 2.3.1). Sample sites are described below and summarized in Tables 2.3.1 and 2.3.2. All samples were analyzed for Cl⁻, NO₃⁻, and ClO₄⁻ concentrations and a subset was evaluated for NO₃⁻ stable isotopic composition as described below.

2.3.2 Site and Sample Descriptions

2.3.2.1 United States

Mojave Desert-Soil

Near surface soil samples (composites) from areas of desert pavement and subsoil samples from discrete depths were collected in the northern Mojave Desert near the U.S. Geological Survey (USGS) Amargosa Desert Research Site (Figure 2.3.1) (Andraski et al., 2014). The Amargosa Desert, in the Basin and Range Province, is bounded by block-faulted mountains composed of Paleozoic metamorphic rocks and Tertiary volcanic rocks. Moderate to steep sloping alluvial fans near the foot of the mountains and the valley floor have sparse, mixed vegetation dominated by creosote bush (Larrea tridentata). Discrete-depth soil and/or salt-rich caliche were also
collected from three sites (Bully Hill, Confidence Hills, Saratoga Hills) in the southern Death Valley region. The Death Valley sites are unvegetated clay hills formed from steeply tilted sedimentary beds and were previously studied for their unusual surface concentrations of NO$_3^-$ and more recently for co-occurring ClO$_4^-$ (Ericksen et al., 1988; Böhlke et al., 1997; Michalski et al., 2004; Jackson et al., 2010; Lybrand et al., 2013). We also obtained archived (filtered and aerated) soil leachates from previous studies of indigenous NO$_3^-$ in the Rainbow Hills and Fort Irwin Basin in the western Mojave Desert (Densmore and Böhlke, 2000; Böhlke et al., 1997). The Mojave Desert sites receive variable rainfall but generally less than 10 cm/year.

**Edwards Aquifer-Groundwater**

Groundwater samples were collected from the San Antonio segment of the fractured karstic Edwards aquifer in Texas, (U.S.) as part of the USGS National Water-Quality Assessment Program (Figure 2.3.1) (Musgrove et al., 2010). Groundwater samples were collected in accordance with procedures described in Koterba and others (1995) and in the USGS National Field Manual. Water samples were collected from public-supply wells in 2004-05 and from a combination of public, domestic, stock, and commercial supply and monitoring wells in 2006. Public-supply well depths ranged from about 65 to 716 m; domestic, commercial, and stock well depths ranged from 24 to 152 m; and monitoring-well depths ranged from 55 to 98 m. The climate is subtropical sub-humid. Mean annual precipitation decreases across the region from 86 cm/year in the east to 56 cm/year in the west (Bomar, 1994). Groundwater from the sampled portion of the Edwards aquifer was recharged predominantly within approximately the past 50 years and mixed extensively in the subsurface, and the whole aquifer is susceptible to anthropogenic impacts (Musgrove et al., 2010).

**Albuquerque Basin-Groundwater**

In the Albuquerque (ABQ) Basin, New Mexico, U.S., groundwater samples were collected from public-supply wells in 2005 and from public-supply and monitoring wells in 2007-2009 (Figure 2.3.1). Public-supply well depths ranged from about 65 to 630 m and monitoring-well depths ranged from about 80 to 360 m. Groundwater samples were collected in accordance with USGS procedures cited above. The climate is semiarid, with potential evaporation substantially exceeding mean annual precipitation (22.1 cm/year at Albuquerque during 1914-2010). The ABQ alluvial basin consists largely of unconsolidated to moderately consolidated deposits of sand, gravel, silt, and clay. The age of most groundwater in the basin is on the order of thousands of years (Plummer et al., 2004; Bexfield et al., 2011).

2.3.2.2 **Southern Africa-Soil and Groundwater**

Soil samples collected in southern Africa were primarily from the hyper-arid Central Namib gravel plains (Figure 2.3.1). Samples represent composites (0-30 cm depth) from locations in an area covering ~150 km north to south and up to 85 km west to east from the coast. Rainfall for all sites is below 10 cm/year and may be as low as 0 cm/year. The eastern-most soil samples were from a calcrete-rich substrate and include the Zebra Pan, a small recharge playa. The central and western soil samples were from gypcrete-rich pediment characterized by lag gravel,
fog precipitation, and lichen growth, and they include a sample from Eisfeld, a saline discharge spring and playa. Samples from Goanikontes were taken at a 20 m high escarpment and include top (A), middle (B) and bottom (C) slopes. A small set of Namibia water samples (springs, potholes, and one groundwater sample) were also obtained from the same region. Additional soil samples were obtained from saline playa surfaces in South Africa (Kalahari Desert; Haskenpan, Koppieskraal and Nolokei) and in Botswana (Makgadikgadi).

2.3.2.2 United Arab Emirates (UAE)—Soil and Groundwater
Soil and groundwater samples were collected from a number of sites in the UAE (Emirate of Abu Dhabi). Soil-surface (0-30 cm) composite samples were from undisturbed areas and discrete-depth samples were from two hand-dug pits in the coastal Sabkha. Groundwater samples were collected from production wells (n=12), monitoring wells (n=3), and shallow hand-dug pits (n=2). Groundwater samples were collected from wells near agricultural areas of Mohayer, Ghayathi, and Liwa. Soil samples were collected from the coastal Sabkha, Matti, and Gayathi regions. The climate is subtropical arid. Rainfall varies across the UAE with reported modern (1966-1998) rainfall ranging from 7-13 cm/year, with a high potential evaporation rate of about 2-3 m/year (Sherif et al., 2014).

2.3.2.5 Atacama Desert—Soil/Caliche
Surface-soil samples (0-30 cm composites) were collected along an elevational transect east of Antofagasta, Chile, extending from the Baquedano nitrate mining district in the ‘absolute desert’ (~1300 m) (i.e., a plant-free landscape) to the tussock steppe grasslands of the Western Andes Cordillera (4200 m) (Figure 2.3.1). Discrete-depth samples were collected from existing or fresh hand excavated pits within 25-75 km of the coast along a 450 km north-south transect. Additional discrete-depth samples were collected from open faces within an active nitrate mine. Atacama NO₃⁻ mineral deposits are considered to have accumulated over a period of the order of 10⁶ to 10⁷ years (Pérez-Fodich et al., 2014). The Atacama Desert is a hyperarid cold desert with estimates of average annual rainfall less than 0.01 cm/year with large stretches of absolute desert.

2.3.2.6 China—Soil and Groundwater

**Turpan-Hami-Caliche**
Caliche-type salt-rich samples were obtained from three sub-basins (Kumutage, Wuzongbulake, and Xiaocahu) in the Turpan-Hami Depressions, an area of fault-bounded troughs that descend to below sea level in northwestern China (Figure 2.3.1). Samples from Kumutage are from a vertical profile (0-2 m depth), whereas samples from the other basins are from unknown depths. These sites are associated with recently described massive NO₃⁻ deposits (Qin et al., 2012). The NO₃⁻ from this region has a wide range of stable isotopic composition, ranging from predominantly un-cycled atmospheric to predominantly biogenic NO₃⁻. The deposits are estimated to be < 260 k years old (Qin et al., 2012). Climate in the region is hyper-arid, with precipitation < 1.5 cm/year.
**Loess Plateau-Groundwater**
Archived (filtered and refrigerated) water samples were obtained from a previous study evaluating groundwater recharge in the central Loess Plateau in Shaanxi Province, China (Gates et al., 2011). Samples were originally collected in 2008. The sampled area has an elevation of ~1,000-2,000 m above sea level. Approximately 20% of the catchment land area is cultivated (primarily dryland wheat farming). Mean annual rainfall is ~50 cm/year. Groundwater is > 100 m deep in upland areas and discharges into incised valleys. The samples were collected from springs over a range of elevations (1,036-1,231 m).

**Badain Jaran-Groundwater**
Archived (filtered and refrigerated) groundwater samples were obtained from a previous study of shallow wells and springs located near the margin between the Badain Jaran dune field and Yabulai Mountains, Gurinai Grass Land, and Xugue Lake area (Figure 2.3.1) (Gates et al., 2008a,b). The area is sparsely settled and contains only limited vegetative cover on unconsolidated sand dunes interspersed with groundwater-fed lakes of varying salinity. The dune area is underlain by a shallow surficial aquifer that is locally confined in some areas. Previous studies indicate groundwater recharge occurs largely at higher elevations near the margins of the basin. The area is considered a cold continental desert with average (1956-1999) precipitation of 8.4 cm/year (Gates et al., 2008a).

**2.3.2.7 Antarctica-Soil**
Discrete depth samples were collected from shallow hand dug pits in University Valley (1600m), which is a perched valley above Beacon Valley in the Quartermain Range of the McMurdo Dry Valley (MDV) region of Antarctica (Figure 2.3.1). University Valley and nearby valleys contain elevated concentrations of NO$_3^-$ primarily of atmospheric origin (Michalski et al., 2005) as well as elevated concentrations of ClO$_4^-$ (Kounaves et al., 2010). University Valley is located in a hyper-arid region of the MDV and receives a current water equivalent precipitation of less than 5 cm/year.
Figure 2.3.1. Map of Sample Locations
Table 2.3.1. Locations, sample types, summary statistics for ClO$_4^-$, NO$_3^-$, and Cl$^-$ in groundwater and deposition sample sets. Molar ratios are averages of log-transformed ratios. r and P values are for linear regressions (see Figure 2.3.2)

<table>
<thead>
<tr>
<th>Location</th>
<th>Site</th>
<th>Sample Type</th>
<th>Molar Ratio</th>
<th>NO$_3$ and ClO$_4$</th>
<th>Cl and ClO$_4$</th>
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Deposition

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58
Table 2.3.2. Locations, sample types, summary statistics for ClO$_4^-$, NO$_3^-$, and Cl$^-$ in soil/caliche sample sets. Molar ratios are averages of log-transformed ratios. R and P values are for linear regressions (see Figure 2.3.5).

<table>
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<th>Sample Type</th>
<th>Sample Depth Range (m)</th>
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<th>Cl/NO$_3$</th>
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2.3.3 Materials and Methods

2.3.3.1 Soil Extraction Methods

Soluble salts from soil samples were extracted by mixing the soil with Milli-Q water at a 5:1 ratio (mass of water: mass of soil) and shaking for 24 h. The samples were centrifuged for 10 minutes, after which the supernatant was decanted and filtered (0.2 µm). All extraction sets were accompanied by an extraction duplicate and extraction sample spike (soil + known amount of ClO$_4^-$ spike), extraction blank (DDI water only), and extraction spike (known amount of ClO$_4^-$ spike). Moisture contents of samples were determined by drying at 105 °C for 24 hours. Subsamples of some extracts were preserved at pH~11 for analysis of NO$_3^-$ stable isotopic composition.

2.3.3.2 Analytical Methods

Analysis of soil extracts and groundwater for ClO$_4^-$, NO$_3^-$, Cl$^-$, and NO$_3^-$ stable isotopic composition has been described previously (Section 2.2; Jackson et al., 2010). Briefly, ClO$_4^-$ was quantified using an ion chromatograph-tandem mass spectrometry technique (IC-MS/MS) that consisted of a GP50 pump, CD25 conductivity detector, AS40 automated sampler and Dionex IonPac AS16 (250 X 2 mm) analytical column. The IC system was coupled with an Applied Biosystems – MDS SCIEX API 2000\textsuperscript{TM} triple quadrupole mass spectrometer equipped with a Turbo-IonSpray\textsuperscript{TM} 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. A 25 µL loop was used for sample loading with a 0.0005 µM level of quantification. Chloride and NO$_3^-$ were analyzed following EPA Method 300.0 using a Dionex LC20, an IonPac AS14A column (4 X 250 mm), 8 mM Na$_2$CO$_3$/1 mM NaHCO$_3$ eluent, and an Anion Atlas electrolytic suppressor. The limit of detection (LOD) of Cl$^-$ and NO$_3^-$were 1.4 µM and 3.5 µM, respectively. Individual sample quantification limits were based on the final dilution of the sample extract.

δ$^{15}$N and δ$^{18}$O in NO$_3^-$ were measured by continuous-flow isotope-ratio mass spectrometry on N$_2$O produced from NO$_3^-$ by bacterial reduction (Sigman et al., 2001, Casciotti et al., 2002, Coplen et al., 2004). The data were calibrated by analyzing NO$_3^-$ isotopic reference materials as samples and normalizing to δ$^{15}$N and δ$^{18}$O reference values (Böhlke et al., 2003; Jackson et al., 2010). For samples with elevated Δ$^{17}$O of NO$_3^-$, δ$^{15}$N values determined by the bacterial N$_2$O method using conventional normalization equations may be slightly higher than true values (Sigman et al., 2001; Böhlke et al., 2003; Coplen et al., 2004). δ$^{15}$N values reported here were not adjusted for this effect because Δ$^{17}$O values were not measured in all samples. True δ$^{15}$N values could be lower than reported values by varying amounts ranging from 0 ‰ for biogenic NO$_3^-$ with δ$^{18}$O and Δ$^{17}$O near 0 ‰ to around 1.6 ‰ for atmospheric NO$_3^-$ from Antarctica with the highest δ$^{18}$O and Δ$^{17}$O values.

For selected samples, the Δ$^{17}$O value of NO$_3^-$ was measured by dual-inlet isotope-ratio analysis of O$_2$ produced by off-line partial decomposition of AgNO$_3$ (modified from Michalski et al.,
NO$_3^-$ was isolated from mixed salt solutions by trapping on large-volume AG1X8 ion-exchange resin columns, followed by gradual elution with 0.5 M KCl to separate anions (Hannon et al., 2008). The KCl-KNO$_3$ eluent was passed through AG-MP50 cation-exchange resin columns in the Ag form to remove Cl and exchange K for Ag, then freeze dried to produce AgNO$_3$ salt. The AgNO$_3$ was heated under vacuum at 520°C while connected to a 5Å mol-sieve trap cooled with liquid N$_2$ to collect O$_2$, which was then isolated and transferred to the mass spectrometer and analyzed against tank O$_2$. Measured Δ$^{17}$O values of NO$_3^-$ isotopic reference materials RSIL-N11 and USGS35 prepared as AgNO$_3$ were indistinguishable from reported values of -0.2 and +21.1 ‰, respectively, as defined in Böhlke et al. (2003).

2.3.4 Data Analysis

Average molar ratios were calculated by averaging log-transformed individual sample ratios and then back transforming to standard notation. This was done to reduce the impact of individual samples with order of magnitude differences in ratios. Regression analysis was performed on data as presented in figures and significance was designated as P < 0.05.

2.3.5 Results and Discussion

Results are summarized for all samples in Figures 2.3.2 and 2.3.3 and Tables 2.3.1 and 2.3.2 to highlight major global patterns and processes affecting the distribution of ClO$_4^-$ and NO$_3^-$. Results for individual sites and sample types are then presented in more detail in Figures 2.3.4 and 2.3.5 to facilitate discussion of local patterns, controls, and exceptions to the global patterns.

2.3.5.1 Global Patterns of ClO$_4^-$, NO$_3^-$ Concentrations and NO$_3^-$ Isotopes

Combined data from this study indicate the global occurrence of ClO$_4^-$ and positive correlation between NO$_3^-$ and ClO$_4^-$ concentrations in oxic groundwaters (Figures 2.3.2, Table 2.3.1), and in soils and caliches (Figure 2.3.3, Table 2.3.2), in arid and semi-arid environments. Previous work has largely attributed the source of indigenous natural ClO$_4^-$ to atmospheric production and subsequent deposition (Section 2.1 and 2.2; Rajagopolan et al., 2009; Jackson et al., 2010). Excluding the Atacama site, our results are generally consistent with this assertion based on similarities between site NO$_3^-$/ClO$_4^-$ ratios and those previously reported. Atmospheric deposition data include wet deposition at 18 sites located across the conterminous U.S., Alaska, and Puerto Rico using weekly samples collected over 3 years (Rajagopolan et al., 2009) and total atmospheric deposition (wet plus dry including dust) collected over a 6 year period at the Amargosa Desert Research Site, Nevada (Andraski et al., 2014). The average molar ratios of NO$_3^-$/ClO$_4^-$ in wet deposition ranged over ~one order of magnitude, while Cl$^-$/ClO$_4^-$ ratios varied over 2 orders of magnitude and were significantly related to distance from a coast. NO$_3^-$/ClO$_4^-$ and Cl$^-$/ClO$_4^-$ molar ratios in total deposition were lower than in wet deposition and the NO$_3^-$ was largely unaltered atmospheric, based on stable isotope ratios (Figures 2.3.2, 2.3.3, 2.3.6; Table 2.3.1).

In contrast to the relatively good correlation between NO$_3^-$ and ClO$_4^-$ at most sites, concentrations of Cl$^-$ appear to be relatively poorly correlated with either NO$_3^-$ or ClO$_4^-$ for most sites (Figures 2.3.4 and 2.3.5). Most samples had relatively high Cl$^-$/NO$_3^-$ and Cl$^-$/ClO$_4^-$ ratios.
compared to those of modern precipitation and total deposition in North America (Figure 2.3.7). Local high deposition fluxes of marine (seasalt) Cl\(^{-}\) could account for some, but not all of these differences. Additional sources of Cl\(^{-}\) unrelated to the Cl\(^{-}\) deposited with ClO\(_4\)- and NO\(_3\)- could include local Cl\(^{-}\) atmospheric inputs remobilized from salt flats, upwelling of saline groundwater, and run-on of Cl\(^{-}\) laden water. Alternatively, high Cl\(^{-}\)/NO\(_3\)- and Cl\(^{-}\)/ClO\(_4\)- ratios could indicate simultaneous net loss of NO\(_3\)- and ClO\(_4\)- as a result of microbial reduction where moisture and organic matter were present (Nozawa-Inoue et al., 2005). This could have occurred during episodic events, defined as locally reducing conditions during individual precipitation wetting events that led to the depletion of oxyanions during the accumulation period of the current oxyanions. Cl\(^{-}\) remaining after such episodic reduction is hereafter referred to as “episodic Cl\(^{-}\)”. Alternatively, oxyanions could have been depleted relative to Cl\(^{-}\) during prolonged periods of wetter climate in the past prior to the accumulation period of the observed oxyanions. For this case, we hereafter refer to atmospheric Cl\(^{-}\) not associated with the current NO\(_3\)- and ClO\(_4\)- as “non-co-deposited Cl\(^{-}\)”. Isotope data indicate sources of NO\(_3\)- from these sites varied from essentially 100% unaltered atmospheric to 100% biogenic, where biogenic NO\(_3\)- could be derived from various N sources including recycled atmospheric NO\(_3\)- (Figure 2.3.6). Collectively, isotopic analyses of NO\(_3\)- are consistent with two major factors affecting \(\delta^{15}N\) and \(\delta^{18}O\) of NO\(_3\)-: (1) variation in the relative proportions of atmospheric NO\(_3\)- (low \(\delta^{15}N\), high \(\delta^{18}O\)) and biogenic NO\(_3\)- (high \(\delta^{15}N\), low \(\delta^{18}O\)), yielding an overall negative correlation between \(\delta^{15}N\) and \(\delta^{18}O\), and (2) variation in the isotope fractionation effect of NO\(_3\)- reduction, yielding local positive correlations between \(\delta^{15}N\) and \(\delta^{18}O\) at some sites. NO\(_3\)- reduction can occur in the unsaturated zone and/or in groundwater after recharge. In addition, minor variations in \(\delta^{18}O\) of biogenic NO\(_3\)- may be related to local \(\delta^{18}O\) of meteoric water during nitrification, and N cycling in soils and plants can cause local or regional variation in the \(\delta^{15}N\) values of organic matter that is subject to nitrification (Handley et al., 1999; Amundson et al., 2003; McMahon and Böhlke, 2006). Isotopic variations caused by N cycling and NO\(_3\)- reduction effects appear to be most prevalent at sites with relatively large fractions of biogenic NO\(_3\)-, such that the combined effects of these major processes give a roughly triangular shape to the collection of isotope data in Figure 2.3.6 with the exception of Antarctica, which has anomalously low \(\delta^{15}N\).

Clearly, patterns and processes are complex and each site is unique. Nevertheless, to facilitate discussion of individual sites and sample types, we present the following conceptual model relating relative concentrations of ClO\(_4\)- and NO\(_3\)- with the isotopic indicators of NO\(_3\)- sources, based on the assumption that atmospheric sources ultimately are important for both ClO\(_4\)- and NO\(_3\)-. Assuming no net gains or losses of ClO\(_4\)- other than atmospheric deposition, relations between NO\(_3\^-/\)ClO\(_4\^-\) ratios and NO\(_3\^-\) \(\delta^{18}O\) values can be explored using a mixing model with three bounding cases (Figure 2.3.8): 1) deposition and preservation of atmospheric NO\(_3\)- with no addition of biogenic NO\(_3\)-; 2) preservation of atmospheric NO\(_3\)- with addition of biogenic NO\(_3\)- produced by nitrification of reduced N from either fixed N\(_2\), atmospheric NH\(_4\)+, or accumulated organic matter (Figure 2.3.8 black dashed lines); and 3) assimilation of atmospheric NO\(_3\)- and regeneration with biogenic NO\(_3\)- with no net gain or loss of N (Figure 2.3.8 red dashed lines). In
this model, we have excluded the case of significant simultaneous NO$_3^-$ and ClO$_4^-$ reduction or NO$_3^-$ reduction alone, which are alternative possibilities discussed below. For the Mojave and Atacama sites, ClO$_4^-$ stable isotope data can be used to exclude large amounts of biological reduction (Jackson et al., 2010). We have not ruled out the possibility of abiotic O exchange with H$_2$O as an alternative to nitrification as a way of lowering $\delta^{18}$O of atmospheric NO$_3^-$, but there is no direct evidence for such exchange in these environments, whereas there is evidence that lower NO$_3^-$ $\delta^{18}$O values are associated with increasing soil biologic activity.
Figure 2.3.2. Relations among ClO₄⁻, NO₃⁻, and Cl⁻ concentrations in groundwater. Ratios for all groundwater samples are shown by solid black lines. Average ratios in U.S. wet deposition are shown as solid red lines, and minimum and maximum average site ratios are shown as dashed lines. Ratios for wet deposition include 18 sites located across the conterminous U.S., as well as Alaska, and Puerto Rico for weekly samples over a three year period (Rajagopolan et al., 2009). The total deposition ratio (green line) represents the 6 year average of quarterly samples (Andraski et al., 2014).
Figure 2.3.3. Relations between concentrations of ClO₄⁻ and NO₃⁻ and Cl⁻ for all soils and caliches. Average ratio in wet deposition is shown as solid red lines, and average minimum and maximum ratios for all sites are shown as dashed lines. Ratios for wet deposition include 18 sites located across the conterminous U.S., Alaska, and Puerto Rico for weekly samples over a three year period (Rajagopolan et al., 2009). The total deposition ratio (green line) represents the 6 year average of quarterly samples (Andraski et al., 2014).
Figure 2.3.4. Relations among ClO₄⁻, NO₃⁻, and Cl⁻ in groundwater from arid and semi-arid locations. Solid black lines represent linear regressions of concentration data; red lines represent averages of log-transformed ratios. Correlation coefficients and P values are listed in Table 2.3.1.
Figure 2.3.5. Relations among ClO₄⁻, NO₃⁻, and Cl⁻ concentrations in soils and caliches from arid and semi-arid locations. Solid black lines represent linear regressions of concentration data; red lines represent averages of log–transformed ratios. Correlation coefficients and P values are listed in Table 2.3.2.
Figure 2.3.6. Relation between $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$ in groundwater and leachate from soil and caliche samples. Black arrows represent the range of slopes reported previously for isotopic fractionation due to denitrification and grey arrows represent general trends in mixing ratios of atmospheric and biogenic NO$_3^-$. 
Figure 2.3.7. Variation of (A) NO$_3$/ClO$_4$ or (B) Cl/ClO$_4$ ratios with respect to Cl/NO$_3$ ratios in soils and groundwaters from arid and semi-arid locations as well as wet and dry deposition in North America. Box plots represent the 25$^{th}$ and 75$^{th}$ percentile values and extended lines from boxes represent the 90$^{th}$ and 10$^{th}$ percentiles. Arrows indicate hypothetical qualitative trajectories that could be caused by various processes. (1Andraski et al., 2104; 2Plummer et al., 2006; 3Rajagopalan et al., 2006).
Figure 2.3.8. Relation between $\delta^{18}O$ of NO$_3^-$ and bulk NO$_3$/ClO$_4$ ratios in soils and groundwaters from arid and semi-arid locations as well as wet and dry deposition in North America. Box plots represent the 25th and 75th percentile values and extended lines from boxes represent the 90th and 10th percentiles. Dashed curves originating at total deposition indicate hypothetical trends caused by either: 1 (red lines) atmospheric NO$_3$ assimilation, mineralization, and nitrification with no net loss or gain of N; or 2 (black curve) addition of biogenic NO$_3$ to uncycled atmospheric NO$_3$, assuming in both cases ClO$_4$ is unaffected. Arrows in the lower left corner indicate hypothetical qualitative trajectories that could be caused by various processes. (\textsuperscript{1}Andraski et al., 2014; \textsuperscript{2}Plummer et al., 2006).
Groundwater Results

Groundwater Overview
The ClO$_4^-$ concentrations in groundwater samples varied over four orders of magnitude, whereas the NO$_3^-$/ClO$_4^-$ molar ratios were remarkably constant (average = 24,000) and almost identical to that of total atmospheric deposition in the Amargosa Desert (Figure 2.3.2). This is true despite the geographic diversity of arid and semi-arid locations and isotopic evidence for varying degrees of biologic N cycling in soils or plants prior to recharge at these sites (Figure 2.3.6 and 2.3.8). Isotopic evidence for varying NO$_3^-$ reduction in groundwater was not associated with substantial variation of NO$_3^-$/ClO$_4^-$ ratios. However, it should be noted that the variation in NO$_3^-$/ClO$_4^-$ ratios within most sites could be sufficient to accommodate substantial (>50%) NO$_3^-$ losses. Cl$^-$/ClO$_4^-$ ratios were much more variable and for most sites these constituents were not correlated (Figure 2.3.2; Table 2.3.1). The lack of correlation and much larger Cl$^-$/ClO$_4^-$ ratios compared to total deposition for non-coastal sites supports addition of Cl$^-$ from non-atmospheric sources, “episodic Cl$^-$” or “non-co-deposited Cl$^-$” in many cases.

Albuquerque Basin, U.S. (ABQ)
ClO$_4^-$ concentrations in ABQ groundwater samples (n=63) varied over a relatively narrow range (< 0.05 (n=22) to 3.1 µg/L) and concentrations were similar to those previously reported for this aquifer system (Plummer et al., 2006) (Figure 2.3.4). While some wells may have been influenced by anthropogenic activities, many were not impacted based on groundwater ages (Plummer et al., 2006; Bexfield et al., 2011). For the 41 samples with detectable ClO$_4^-$, NO$_3^-$ and ClO$_4^-$ were correlated (r = 0.82) but there was no correlation between Cl$^-$ and either ClO$_4^-$ or NO$_3^-$ (r = 0.22 and 0.05, respectively) (Table 2.3.1 and Figure 2.3.4). The average NO$_3^-$/ClO$_4^-$ and Cl$^-$/ClO$_4^-$ molar ratios were 22,000 and 300,000, respectively.

A subset (n= 30) of ABQ groundwater samples was also evaluated for NO$_3^-$ stable isotopic composition ($\delta^{15}$N and $\delta^{18}$O) (Bexfield et al., 2011). NO$_3^-$ in the aquifer appears to be almost completely biogenic and NO$_3^-$ in a number of wells may have been impacted by denitrification given the linear trend, correlation (r = 0.90) and slope (1.05) of some $\delta^{15}$N and $\delta^{18}$O data pairs (Figure 2.3.6) (Granger et al., 2008). These results are consistent with previous findings (Plummer et al., 2006). NO$_3^-$ stable isotope data were available for 9 of the 22 samples with ClO$_4^-$ concentrations below the reporting limit (0.05 µg/L). Of these, the $\delta^{15}$N and $\delta^{18}$O values of all but one exhibited evidence of denitrification. These data could indicate ClO$_4^-$ reduction was associated with partial NO$_3^-$ reduction in some parts of the system. For water samples (n = 21) with detectable ClO$_4^-$ and NO$_3^-$, and with NO$_3^-$ stable isotope data, $\delta^{18}$O values of NO$_3^-$ were relatively low (< 1 %o for all but 2 samples) and there was no apparent relation between NO$_3^-$/ClO$_4^-$ molar ratios and $\delta^{18}$O or $\delta^{15}$N values that would indicate substantial NO$_3^-$ reduction or ClO$_4^-$ reduction. Dissolved O$_2$ concentrations were highly variable (< 0.1-7 mg/L) but there was no apparent relationship with NO$_3^-$ or ClO$_4^-$ concentrations. Jackson et al., (2010) reported
similar NO$_3^-$ isotopic composition ($\delta^{18}O \sim -1\%$) and NO$_3^-$/ClO$_4^-$ ratios (18,000 and 28,000) for two oxic groundwater samples from the ABQ basin.

NO$_3^-$/ClO$_4^-$ ratios and NO$_3^-$ stable isotopic compositions of ABQ groundwaters are consistent with a model of atmospheric deposition and subsequent recycling of NO$_3^-$ with no net addition of NO$_3^-$ (Figure 2.3.8), but with substantial addition of Cl$^-$ (Figure 2.3.7). The source of the Cl$^-$ could originally be from non-atmospheric sources, “episodic Cl$^-$”, or “non-co-deposited Cl$^-$” (as previously defined). For the “episodic Cl$^-$” case, NO$_3^-$ and ClO$_4^-$ would have to have been reduced substantially (~2 orders of magnitude), which is not supported by available isotope data for NO$_3^-$ (Figure 2.3.6) or ClO$_4^-$ (Jackson et al., 2010).

**Edwards Aquifer, U.S.**

ClO$_4^-$ concentrations in Edwards Aquifer samples (n= 92) varied over a narrow range (< 0.05 (n = 2) to 1.0 µg/L). No information is available regarding the source of the ClO$_4^-$ in this system and it is possible that some groundwater is impacted by anthropogenic NO$_3^-$ and ClO$_4^-$. NO$_3^-$ and ClO$_4^-$ were marginally correlated ($r = 0.22$), as were Cl$^-$ and ClO$_4^-$ ($r = 0.27$) but no significant relation exists between Cl$^-$ and NO$_3^-$ (Table 2.3.1 and Figure 2.3.4). The average NO$_3^-$/ClO$_4^-$ molar ratio (22,000) was the same as that for the ABQ basin, but the average Cl$^-$/ClO$_4^-$ molar ratio (96,000) was less than that for the ABQ basin. The stable isotopic compositions of NO$_3^-$ are not available for samples evaluated for ClO$_4^-$ concentrations. Other studies have evaluated the stable isotopic composition of NO$_3^-$ in the same segment of the Edwards Aquifer and concluded that the NO$_3^-$ is predominately biogenic with no evidence of denitrification based on either the isotope data or excess N$_2$ gas (Musgrove et al., 2010). Similar to the ABQ basin, the data could be consistent with a model in which atmospheric NO$_3^-$ deposition largely was replaced by biogenic NO$_3^-$ in recharge with no net loss or gain, and with addition of Cl$^-$ (~10X) from sources other than atmospheric co-deposition (Figure 2.3.7).

**Loess Plateau, China**

ClO$_4^-$ concentrations in Loess Plateau groundwater samples (n=10) varied over a range (0.6-1.6 µg/L) similar to those for the ABQ basin and Edwards Aquifer (Figure 2.3.4). NO$_3^-$ and ClO$_4^-$ concentrations were correlated ($r=0.95$) but Cl$^-$ was not correlated with either ClO$_4^-$ or NO$_3^-$ (Table 2.3.1). The NO$_3^-$/ ClO$_4^-$ and Cl$^-$/ClO$_4^-$ molar ratios were the lowest of any groundwater evaluated (~11,000 and 47,000, respectively). No data are available concerning the age of the groundwater or the origin of the NO$_3^-$ but the NO$_3^-$/ ClO$_4^-$ ratios are less and Cl$^-$/ClO$_4^-$ ratios greater than in measured deposition (Figure 2.3.2 and 2.3.7). Although agriculture was a common land use in the region, sampled groundwater was not highly enriched in NO$_3^-$. Irrigation was almost absent in the area. It is not possible to discuss the origin or subsequent fate of NO$_3^-$ due to the absence of NO$_3^-$ isotope data, but the relation between NO$_3^-$ and ClO$_4^-$ is similar to that in other geographically distant sites.
**Badain Jaran, China**

ClO$_4^-$ concentrations in groundwater samples (n=20) ranged from 0.3 to 2.1 µg/L and were correlated with NO$_3^-$ (r = 0.57) (Figure 2.3.4, Table 2.3.1) but Cl$^-$ was not correlated with ClO$_4^-$ or NO$_3^-$. The average NO$_3^-$/ClO$_4^-$ molar ratio (61,000) was the highest of any of the groundwater locations. Given the remoteness of the location, lack of intensive agriculture, and age of at least some of the groundwater evaluated, it is likely the ClO$_4^-$ is indigenous (Gates et al., 2008a,b). NO$_3^-$ in this system previously has been attributed to nitrification of NH$_4^+$ derived from atmospheric deposition or from mineralization of organic matter produced by nitrogen fixation or biologic assimilation of atmospheric N. In a few samples, NO$_3^-$ may have been partially reduced through denitrification and a few samples with elevated δ$_{18}$O and low δ$_{15}$N of NO$_3^-$ may have minor unaltered atmospheric components (Gates et al., 2008a). Our samples represent a subset of those analyzed by Gates et al. (2008a). Of the 20 samples we evaluated for ClO$_4^-$, NO$_3^-$ stable isotope data were available for 10 (Figure 2.3.6). Six samples previously identified as most likely to contain NO$_3^-$ impacted by denitrification have NO$_3^-$/ClO$_4^-$ molar ratios that are not significantly lower than the overall average (i.e., do not appear to indicate substantial preferential NO$_3^-$ loss). Three samples appear to be impacted by denitrification based on relatively low NO$_3^-$/Cl$^-$ ratios and elevated δ$_{15}$N and δ$_{18}$O values (Gates et al., 2008a). However, even for these samples the ratio of NO$_3^-$/ClO$_4^-$ is not consistently lower than the overall average.

NO$_3^-$ and ClO$_4^-$ occurrence are consistent with atmospheric deposition and at least partial preservation of the atmospheric NO$_3^-$ with substantial dilution (replacement and addition) by biogenic NO$_3^-$ based on slightly elevated δ$_{18}$O and elevated NO$_3^-$/ClO$_4^-$ ratios (Figure 2.3.18). Cl$^-$/ClO$_4^-$ and Cl$^-$/NO$_3^-$ ratios support large (~100X) inputs of local, “episodic”, or “non-co-deposited atmospheric Cl-$^-$, similar to the ABQ aquifer (Figure 2.3.7).

**Namibia**

ClO$_4^-$ concentrations in Namibia groundwater samples (n = 10) ranged from 2.7-97 µg/L (Figure 2.3.4). ClO$_4^-$ was correlated with NO$_3^-$ and Cl$^-$ (r = 0.91 and 0.89, respectively) and as such NO$_3^-$ and Cl$^-$ were also correlated (r=0.96) (Table 1). Given the remote location of the sample sites, high concentrations of ClO$_4^-$ and NO$_3^-$, and NO$_3^-$ isotopic composition, the ClO$_4^-$ and NO$_3^-$ are considered to be natural. The average NO$_3^-$/ClO$_4^-$ ratio (29,000) was similar to those in all other groundwater samples evaluated excluding the Badain Jaran, while the average Cl$^-$/ClO$_4^-$ molar ratio was much higher (2,300,000). Stable isotope data indicate NO$_3^-$ in the Namibia samples was predominantly biogenic, but included substantial unaltered atmospheric components based on elevated Δ$_{17}$O values in some samples (1.1 to 5.9 ‰; n = 5). The NO$_3^-$ also appears to have been variably affected by NO$_3^-$ reduction based, on elevated δ$_{18}$O and δ$_{15}$N values (Figure 2.3.6) and higher δ$_{18}$O values than expected from biogenic-atmospheric mixtures based on the Δ$_{17}$O values.

Factors affecting the Namibia groundwater could be similar to those at the Badain Juran site (atmospheric deposition with conservation of the atmospheric component and dilution by
biogenic NO$_3^-$ but altered by some denitrification (Figure 2.3.8). High Cl$^-$/ClO$_4^-$ ratios could be due to high local Cl$^-$ deposition fluxes considering the coastal proximity, as values overlap with the high end of the range for wet deposition from US coastal locations. This is supported by the significant correlation between Cl$^-$ and ClO$_4^-$ for this site but not others, and by the similar Cl$^-$/ClO$_4^-$ ratios in surface soils (Figure 2.3.7).

**UAE**

Maximum ClO$_4^-$ concentrations for UAE groundwater were the highest of any groundwater evaluated (Figure 2.3.4). Highest concentrations (500-740 µg/L) occurred in sabkha areas (n = 5) but concentrations in some inland fresh groundwater (n=20) were also elevated (0.1 to 108 µg/L), similar to the concentration range for Namibia groundwater. ClO$_4^-$ and NO$_3^-$ were well correlated with each other ($r = 0.98$) and with Cl$^-$ (Table 1). The average NO$_3^-$/ClO$_4^-$ molar ratio (22,000) was similar to those for the Edwards, ABQ, and Namibia sites (22,000, 23,000, and 29,000, respectively) (Table 2.3.1). The NO$_3^-$ in most (n=10) of the fresh-water samples (production wells) for which NO$_3^-$ stable isotope data are available (n=12) appears to be predominantly biogenic, with little evidence of NO$_3^-$ reduction ($\delta^{15}N \sim 2 - 6 \%$ and $\delta^{18}O \sim 5 - 10 \%$; Figure 2.3.6). In contrast, all of the sabkha locations and two of the fresh-water well samples had a substantial unaltered atmospheric component with variable impacts from NO$_3^-$ reduction based on $\delta^{18}O$ and $\delta^{15}N$ of NO$_3^-$ (Figure 2.3.6) as well as $\Delta^{17}O$ of NO$_3^-$ (8 to 8.5‰) in a subset of the sabkha samples.

The sabkha data are consistent with evapo-concentration of deposition with some dilution by biogenic NO$_3^-$ and only limited loss of the atmospheric NO$_3^-$ component if the impact of NO$_3^-$ reduction indicated by isotope data is taken into account. Cl$^-$/ClO$_4^-$ ratios were elevated and variable, possibly reflecting variable marine, atmospheric, and subsurface salt sources, and are generally similar to Namibia groundwater values, consistent with the coastal location and correlation between Cl$^-$ and ClO$_4^-$ (Table 2.3.1, Figure 2.3.7). UAE non-sabkha groundwater is more consistent with NO$_3^-$ cycling rather than simple dilution (addition only) by biogenic NO$_3^-$.

**2.3.5.3 Soil and Caliche Results**

**Soil and Caliche Overview**

ClO$_4^-$ concentrations in soils/caliches for all sites vary over a larger range (0.1 to 10$^6$ µg/kg) than those in groundwater (Figure 2.3.2 and 2.3.3). Similarly, NO$_3^-$/ClO$_4^-$ ratios appear to be more variable in soils/caliches than in groundwater (Figure 2.3.2 and 2.3.3). In part, these contrasts may be related to the fact that soil/caliche samples are likely to exhibit local heterogeneity caused by selective crystallization, re-dissolution, and redistribution of salts during infiltration, whereas groundwater is more likely to represent spatially and temporally integrated samples of
infiltrated salts. Nonetheless, there are clear relations between soil and groundwater NO$_3^-$ and ClO$_4^-$ concentrations within sites and between sites.

Overall there appear to be major groupings of NO$_3^-$/ClO$_4^-$ molar ratios that include 1) Mojave soil/caliche (85,000) and southern Africa soil (120,000); 2) all groundwater (24,000) and UAE soil (21,000); 3) Antarctica soil (14,000) and China–Turpan Hami soil/caliche (12,000); and 4) Atacama soils/caliches (1,400) (Figure 2.3.7). The first group (Mojave and southern Africa soil) ratios are similar to the average ratio (~100,000) in wet deposition (Rajagopolan et al., 2009). The ratios of the second group (all groundwaters and UAE soil) are essentially identical to the ratio in total deposition at the Amargosa Desert Research Site (22,000) (Andraski et al., 2014). The third group (Antarctica and Turpan Hami) has lower ratios but are still similar to that in total atmospheric deposition, while the Atacama ratio is an order of magnitude lower and unique. The last two groups, with the lowest NO$_3^-$/ClO$_4^-$ ratio, are also the most arid. Soil Cl$^-$/ClO$_4^-$ ratios are much more variable than NO$_3^-$/ClO$_4^-$ ratios, as in the groundwater sample sets; only the Antarctica and Atacama soil data sets exhibit a clear relation between Cl$^-$ and ClO$_4^-$. Each site is discussed below in more detail in order of the major groupings and generally from more biologically active to less biologically active.

**Mojave Desert**

Soil samples were collected from three different environments (surface of clay hills, valley floor desert pavement, and deep sub-surface salt bulge), all in a relatively small but geographically diverse area of the Mojave Desert (Figure 2.3.1). These samples do not represent “typical” soils but rather were chosen for their known accumulations of salts. Concentrations of ClO$_4^-$ in these samples ranged from a low of 0.1 µg/kg (salt bulge) to a high of 5,500 µg/kg (Confidence Hills) (Figure 2.3.5). Samples (0-1m) from the clay hills of southern Death Valley (Confidence Hills, Bully Hill, and Saratoga Hills) had the highest known concentrations of indigenous ClO$_4^-$ in the U.S., and were similar to those described in previous studies (Jackson et al., 2010; Lydbrand et al., 2013). Discrete-depth samples from a subsurface salt bulge have a wide range of ClO$_4^-$ concentrations (0.1 to 20 µg/kg), similar to other reported southwestern U.S. subsurface salt bulges (Rao et al., 2007). Near-surface samples from desert pavement on the Amargosa Desert valley floor contain concentrations of ClO$_4^-$ similar to those in the nearby subsurface salt bulge. For comparison, a set (n=48) of composite (0-30 cm) samples collected in a pre-defined grid covering a 0.1 km$^2$ hill-slope area of a nearby knoll was reported to have an average ClO$_4^-$ concentration of 3.0 ± 2.6 µg/kg with a range of 0.8 to 11.8 µg/kg (Andraski et al., 2014).

ClO$_4^-$ concentrations for all sites in the Mojave Desert are significantly correlated with NO$_3^-$ ($r = 0.66$) but not with Cl$^-$ ($r = 0.18$) (Table 2.3.2, Figure 2.3.5). ClO$_4^-$ and NO$_3^-$ concentrations from individual profiles in and near the Death Valley clay hills were generally but not always significantly correlated. In addition, for the spatially varying desert pavement composite, desert pavement profile, and subsurface salt bulge, concentrations of ClO$_4^-$ and NO$_3^-$ were correlated ($r$
ClO$_4^-$ was not correlated with Cl$^-$ in the Mojave sample sets except for the subsurface salt bulge, desert pavement sites, and samples from Rainbow Hills. Cl$^-$ and NO$_3^-$ were also correlated for many of the same locations for which ClO$_4^-$ and NO$_3^-$ were correlated. A previous study found a significant but weaker relationship between NO$_3^-$ and ClO$_4^-$ in samples from the clay hills including some of the same locations reported here (Lybrand et al., 2013). One possible reason for the lower degree of correlation in the Lybrand study may have been the relatively high reportable ClO$_4^-$ detection limit (165 µg/kg).

The molar ratios of NO$_3^-$/ClO$_4^-$ for all Mojave locations varied from 25,000 to 240,000 with an overall average of 85,000 (Table 2.3.2). NO$_3^-$/ClO$_4^-$ molar ratios were most variable in the clay hills samples, while the desert pavement and sub-surface salt bulge NO$_3^-$/ClO$_4^-$ ratios were more uniform (40,000 to 86,000). These ratios are similar to those reported for other salt bulges in the arid southwestern U.S. (30,000 - 51,000, 27,000, and 218,000 for depth profiles from the Chihuahuan Desert, Amargosa Desert, and Yucca Flats, respectively) (Rao et al., 2007). Mojave Desert soil NO$_3^-$/ClO$_4^-$ molar ratios are generally higher than the average ratio in total deposition at the Amargosa Desert Research Site (Andraski et al., 2014). The total deposition sample site was co-located with the desert pavement and sub-surface salt-bulge sites and within ~ 100 km of the remaining Mojave sites. In contrast to the relatively similar average NO$_3^-$/ClO$_4^-$ ratios in Mojave sample sets, average Cl$^-$/ClO$_4^-$ ratios varied over two orders of magnitude (77,000 to 7,400,000), indicating processes other than evapo-concentration affected anion ratios in the region.

Stable isotope data for NO$_3^-$ (δ$^{18}$O, Δ$^{17}$O, and δ$^{15}$N) in samples from the Death Valley clay hills and Rainbow Hills previously demonstrated that the NO$_3^-$ is partially (20-50%) atmospheric in origin and partially biogenic, with no evidence of substantial isotope effect from NO$_3^-$ reduction (Böhlke et al., 1997; Michalski et al., 2004; Jackson et al., 2010; Lybrand et al., 2013). NO$_3^-$ in soil samples from Irwin Basin was mainly biogenic (Figure 2.3.6) (Densmore and Böhlke, 2000). The δ$^{18}$O and δ$^{15}$N of NO$_3^-$ in samples from the desert salt bulge support a largely biogenic source of NO$_3^-$ while those in samples from near-by desert pavement appear to have a substantial (up to ~20%) atmospheric component (Figure 2.3.6) (Andraski et al., 2014).

NO$_3^-$/ClO$_4^-$ ratios and NO$_3^-$ stable isotope ratios for samples from the Mojave clay hills are consistent with a model of atmospheric deposition and addition of biogenic NO$_3^-$ without significant recycling (Figure 2.3.8), and with substantial addition of Cl$^-$ (Figure 2.3.7) from “episodic Cl$^-$”, “non-co-deposited Cl$^-$” sources or local non-atmospheric sources, consistent with the sedimentary host rocks and lack of plant life on the hills. Samples from the deep bulge are more similar to groundwater, indicating a largely recycled NO$_3^-$ component consistent with N that infiltrated through the active soil zone. Samples of desert pavement are intermediate to the other two sample sets reflecting limited recycling of the atmospheric NO$_3^-$ component and addition of biogenic NO$_3^-$.
The relatively low \( \text{NO}_3^-/\text{ClO}_4^- \) ratios in the salt bulge and groundwaters may indicate that soil processes tend to decrease \( \text{NO}_3^- \) relative to \( \text{ClO}_4^- \). The decrease in the \( \text{NO}_3^-/\text{ClO}_4^- \) ratio could be due to episodic microbial reduction, though soils in these arid environments are generally well aerated. Another possibility could be uptake and assimilation of \( \text{NO}_3^- \) by plants and accumulation as organic N. A recent study suggested that while both \( \text{ClO}_4^- \) and \( \text{NO}_3^- \) are taken up by desert plants, the \( \text{ClO}_4^- \) can remain unprocessed and recycled back to the soils while \( \text{NO}_3^- \) is largely fixed within the plant tissue (Andraski et al., 2014). The similar low \( \text{NO}_3^-/\text{ClO}_4^- \) ratio for groundwaters and deep salt bulge, given the complete loss of the original atmospheric \( \text{NO}_3^- \) component, suggests that in arid and semi-arid areas there is an upper limit to the amount of oxidized N that escapes from the biologically active vadose zone and that long term fluxes of \( \text{NO}_3^- \) may be somewhat regulated. Only in cases of extreme aridity or surface features (e.g. desert pavement), which limit infiltration, do ratios increase from \( \text{NO}_3^- \) addition in the relative absence of assimilation and gas loss.

**Southern Africa**

In contrast to the Mojave sample sites, southern Africa soil sample sites were not selected based on known salt accumulations. \( \text{ClO}_4^- \) concentrations varied from 0.2 to 45 \( \mu \text{g/kg} \), with no relation to surface feature (pan, fan, or vertical profile) (Figure 2.3.5). Samples from Botswana and South Africa playa surfaces were similar to those from Namibia. For both the Namibia and Botswana sites, as well as the combined data set, \( \text{ClO}_4^- \) and \( \text{NO}_3^- \) concentrations were correlated (Table 2.3.2, Figure 2.3.5). Similar to the Mojave Desert samples, there were no significant correlations between \( \text{Cl}^- \) and \( \text{ClO}_4^- \) or \( \text{Cl}^- \) and \( \text{NO}_3^- \). Average molar ratios of \( \text{NO}_3^-/\text{ClO}_4^- \) were similar for the three sites (96,000, 150,000, and 125,000) with an average ratio of 120,000 (Table 2.3.2), significantly greater than Namibia groundwater samples. Site average \( \text{Cl}^-/\text{ClO}_4^- \) ratios were much more variable (650,000 - 41,000,000). \( \text{NO}_3^- \) in the Namibia surface soils contained a greater unaltered atmospheric \( \text{NO}_3^- \) component (20 - 50%) than groundwater from Namibia (Figure 2.3.6), and was intermediate to Mojave Desert pavement and clay hill samples (Figure 2.3.8). \( \text{NO}_3^-/\text{ClO}_4^- \) ratios largely overlap with ratios of the Mojave Desert sample sets (e.g. clay hills and desert pavement) but are higher than ratios for Namibia groundwater (Figure 2.3.8).

Overall, the Namibia soil data set appears to resemble the Mojave clay hills data set, consistent with a largely preserved atmospheric component with addition of biogenic \( \text{NO}_3^- \) (Figure 2.3.8). While the unusually high \( \text{Cl}^-/\text{ClO}_4^- \) ratios in the Namibia samples could in part be due the coastal proximity, the lack of correlation between \( \text{Cl}^- \) and \( \text{NO}_3^- \) or \( \text{ClO}_4^- \) indicates local, “episodic”, or ‘non-co-deposited \( \text{Cl}^- \)’ sources may be important, similar to the Mojave Clay hills (Figure 2.3.7).

**UAE**

Sample sites in the UAE represent both typical soil and sabkha sediment. Soil \( \text{ClO}_4^- \) concentrations \((n=7)\) ranged from \(<0.1 \ (n=2)\) to 13 \( \mu \text{g/kg} \) and those for sabkha sediment \((n=7)\) were generally higher \((0.1< \ (n = 2)\) to 262 \( \mu \text{g/kg} \)). \( \text{ClO}_4^- \) and \( \text{NO}_3^- \) were correlated for both soil \((r
and sabkha sediments \((r = 0.97)\) with an overall correlation coefficient of 0.99 (Table 2.3.2 and Figure 2.3.5). Cl\(^-\) and ClO\(_4\)\(^-\) were not correlated, nor were NO\(_3\)\(^-\) and Cl\(^-\). NO\(_3\)/ClO\(_4\)\(^-\) molar ratios were similar in soils and sabkha sediments (14,000 and 28,000, respectively), while Cl/ClO\(_4\)\(^-\) ratios were much more variable (19,000 and 1,700,000, respectively). Isotope data indicate the source of NO\(_3\)\(^-\) in the soil samples is mainly biogenic except for two samples that may have around 20-50% unaltered atmospheric NO\(_3\)\(^-\), both of which had very low NO\(_3\)\(^-\) and ClO\(_4\)\(^-\) concentrations (Figure 2.3.6). NO\(_3\)\(^-\) in the sabkha samples apparently had substantial fractions of unaltered atmospheric NO\(_3\)\(^-\) (approximately 20-50 %), and it also exhibited isotopic evidence of NO\(_3\)\(^-\) reduction. NO\(_3\)/ClO\(_4\)\(^-\) molar ratios in UAE soil and sabkha samples were similar to those in UAE groundwater (22,000) and Amargosa Desert total deposition (22,000) (Figure 2.3.8).

The NO\(_3\)/ClO\(_4\)\(^-\) ratios and NO\(_3\)\(^-\) isotopic compositions of the soil samples are consistent with substantial NO\(_3\)\(^-\) recycling, perhaps consistent with relatively high local precipitation, similar to UAE groundwater (Figure 2.3.8). Sabkha samples are more consistent with biogenic addition and limited recycling, given the large unaltered atmospheric component and higher NO\(_3\)/ClO\(_4\)\(^-\) ratios and expected impact of NO\(_3\)\(^-\) reduction. Interestingly, UAE soil Cl/ClO\(_4\)\(^-\) ratios are near Mojave total deposition values and an order of magnitude lower than UAE groundwater values despite the similar NO\(_3\)/ClO\(_4\)\(^-\) ratios and proximity to the coast. The lack of correlation between soil Cl\(^-\) and ClO\(_4\)\(^-\), but strong correlation for groundwater coupled with higher ClO\(_4\)\(^-\) concentrations in groundwater than soil, suggest that the groundwater Cl\(^-\), NO\(_3\)\(^-\), and ClO\(_4\)\(^-\) were not simply derived from concentrated infiltration but rather may indicate dissolution of stored salts in the subsurface or that the groundwater was not recharged locally.

**China, Turpan-Hami**

High concentrations of ClO\(_4\)\(^-\) (~100 to 16,000 µg/kg) were present in a relatively small set \((n = 11)\) of samples from three sites in the vicinity of recently described massive NO\(_3\)\(^-\) deposits of northern China (Qin et al., 2012; Li et al., 2010). Concentrations of ClO\(_4\)\(^-\) were similar to those in the Mojave clay hills samples and ~1-2 orders of magnitude lower than the majority of samples from the Atacama NO\(_3\)\(^-\) deposits, even though the NO\(_3\)\(^-\) concentrations were similar between the Atacama and China deposits (Figure 2.3.5). Turpan-Hami NO\(_3\)\(^-\) has been attributed largely to long-term atmospheric deposition, based on elevated \(\Delta^{17}\)O (5-20‰) and \(\delta^{18}\)O (30-60‰) values (Qin et al., 2012; Li et al., 2010), which are intermediate to NO\(_3\)\(^-\) from the Mojave clay hills and Atacama Desert. Previously reported ranges of \(\delta^{18}\)O and \(\Delta^{17}\)O indicate the Turpan-Hami NO\(_3\)\(^-\) is approximately 25-75% unaltered atmospheric. Data from the current study indicate similar atmospheric components (Figure 2.3.6). NO\(_3\)\(^-\) and ClO\(_4\)\(^-\) were correlated \((r = 0.61)\) but Cl\(^-\) and ClO\(_4\)\(^-\), and Cl\(^-\) and NO\(_3\)\(^-\), were not. Cl\(^-\) concentration varied relatively little, while NO\(_3\)\(^-\) and ClO\(_4\)\(^-\) concentrations varied over 2 orders of magnitude (Figure 2.3.5). Molar ratios of NO\(_3\)/ClO\(_4\)\(^-\) were generally lower than ratios for the Mojave Desert sites and for Mojave total atmospheric
deposition, but the overall average ratio was still ~1 order of magnitude greater than the Atacama ratio, although the ranges do overlap (Figure 2.3.7).

Given the large unaltered atmospheric NO$_3^-$ component and the relatively low NO$_3^-$/ClO$_4^-$ ratio in the Turpan-Hami samples, it would appear that there has not been much net addition of biogenic NO$_3^-$ (Figure 2.3.8). Compared to the Mojave total deposition anion ratios and NO$_3^-$ isotope values, the Turpan-Hami data are consistent with partial recycling of the atmospheric NO$_3^-$ component. However given the extreme aridity and lack of vegetation, an alternative explanation may be that the Mojave total deposition NO$_3^-$/ClO$_4^-$ ratios do not represent Turpan-Hami deposition ratios. For example, a lower contribution from wet deposition with higher NO$_3^-$/ClO$_4^-$ might reduce the total deposition NO$_3^-$/ClO$_4^-$ ratio and thus allow for biogenic addition without recycling. Similar to the Mojave, there also appears to be a substantial component of Cl$^-$ unrelated to co-deposited NO$_3^-$ and ClO$_4^-$ (Figure 2.3.7).

**Antarctica, University Valley**

ClO$_4^-$ concentrations varied over a relatively narrow range (50-500 µg/kg); variations with depth were similar in magnitude to variations among locations (Figure 2.3.5). These concentrations are similar to those previously reported for University Valley and Beacon Valley (Kounaves et al., 2010). Concentrations were higher than those of surface soils from other sites, excluding the samples from the Mojave clay hills, Atacama, and China Turpan-Hami. Concentrations of NO$_3^-$ and ClO$_4^-$ were correlated ($r = 0.83 - 0.99$) in all profiles, as were concentrations of Cl$^-$ with ClO$_4^-$ ($r = 0.72 - 0.99$) and NO$_3^-$ ($r = 0.64 - 0.99$) with the exception of profile 7 (Table 2.3.2). Average molar ratios were similar among sites 7,900 - 20,000; 4,600 - 10,000; and 0.34 - 0.87, for NO$_3^-$/ClO$_4^-$, Cl/ClO$_4^-$, and Cl/NO$_3^-$ respectively. The NO$_3^-$/ClO$_4^-$ molar ratios were similar to those of Turpan-Hami samples, but an order of magnitude higher than Atacama ratios. The Cl$^-$/NO$_3^-$ molar ratios for the Antarctica sites were lower than for all other sites, but the Cl$^-$/ClO$_4^-$ ratios for the Atacama sites were lower than those for University Valley (Figure 2.3.7).

NO$_3^-$ in soils from the McMurdo Dry Valley region of Antarctica previously has been attributed to atmospheric deposition with no biogenic component based on extremely high $\delta^{18}$O ($>70\%o$) and $\Delta^{17}$O values ($>29\%o$) (Michalski et al., 2005). NO$_3^-$ in Antarctica soil samples analyzed in this study had a similar $\delta^{18}$O range (76-84‰) (Figure 2.3.6). This site represents the extreme end of low biological activity both due to the very few degree days above 0°C as well as the limited precipitation. NO$_3^-$ deposition in Antarctica is somewhat unique and NO$_3^-$ can also be subject to a number of isotopically fractionating abiotic processes (e.g. photolysis and volatilization) on snow and ice (e.g. Grannas et al., 2007 and Frey et al., 2009). Our soil samples do not appear to exhibit anomalous concentration ratios or NO$_3^-$ isotopic compositions that might reflect post-depositional transformations. The ratios of NO$_3^-$/ClO$_4^-$ and Cl/ClO$_4^-$, and NO$_3^-$ isotopic compositions, coupled with strong correlations among all three species, support an unaltered
atmospheric source consistent with the cold hyper-arid conditions and almost complete lack of biological activity (Figure 2.3.8).

**Atacama**

ClO$_4^-$ concentrations ($n=102$) in Atacama soil/caliche samples ranged from a minimum of 0.5 µg/kg to a maximum of $1 \times 10^6$ µg/kg (Figure 2.3.5). The vast majority of profile samples (AT) collected in the central depression contained ClO$_4^-$ at concentrations that exceed those for all other sites evaluated in this study. Surface soil composites acquired along an east-west elevation transect vary in concentration; approximately 50% of the samples (those located above 2500 m elevation, east of the central depression and absolute desert where vegetation is present) had concentrations similar to those for the Mojave, southern Africa, and UAE sites. Spatial location (both altitude and geographic coordinates) is likely an important determinant of ClO$_4^-$ concentration due to rainfall and evapotranspiration effects, but the associated discussion is beyond the scope of this paper.

For all profiles, surface composites, and the combined data set, NO$_3^-$ and ClO$_4^-$ were correlated except for the mine samples (Table 2.3.2). In general, correlation coefficients were lower for the relation between Cl$^-$ and ClO$_4^-$ compared to NO$_3^-$ and ClO$_4^-$ but still generally significant, and as such NO$_3^-$ and Cl$^-$ were generally correlated. NO$_3^-/ClO_4^-$ molar ratios (550-2,200) were at least an order of magnitude lower than at other sites evaluated in this study, with one exception (site AT16, where NO$_3^-/ClO_4^-$ = 13,000). All the Atacama NO$_3^-/ClO_4^-$ ratios were below the minimum ratios reported for wet and total deposition in North America (Rajagopalan et al., 2009 Andraski et al., 2014). Previous studies indicate the source of NO$_3^-$ in the Atacama caliche deposits is largely atmospheric (> 50%) (Böhlke et al., 1997; Michalski et al., 2004) and the stable isotopic compositions of samples evaluated in this study generally were consistent with this, except for a few of the surface soil composites far from the central depression in which NO$_3^-$ appears to be mainly biogenic (Figure 2.3.6). Cl$^-/ClO_4^-$ molar ratios for the Atacama sites were similar to each other in magnitude and variability (615 - 4,700) and were lower than at other sites evaluated in this study (Table 2.3.2).

The Atacama Desert is the location with the longest record of aridity and the most extreme aridity under present conditions, and this is reflected by the predominantly atmospheric source of NO$_3^-$ with only limited biogenic NO$_3^-$ (Böhlke et al., 1997; Michalski et al., 2004). The average Atacama soil NO$_3^-/ClO_4^-$ ratio is at least one order of magnitude lower than all other measured deposition, soil/caliche, and groundwater ratios (Figure 2.3.8). We exclude the possibility that the low ratio compared to other sites was caused by loss of ClO$_4^-$ at all other sites based on the stable isotopic composition of ClO$_4^-$ in the Mojave (Jackson et al., 2010) as well as other unpublished data for Antarctica and Turpin-Hami. There was also limited or no evidence for biological reduction in most of the sites studied. The low ratio is not likely caused by net loss of NO$_3^-$ by denitrification or plant assimilation, given the hyper-arid conditions. The low ratio
could be due to regionally low NO$_3^-$ deposition flux or high ClO$_4^-$ deposition flux. Both of these species are considered to be at least partly atmospheric in origin based on elevated $\Delta^{17}$O values as well as $^{36}$Cl content of ClO$_4^-$ (Bao and Gu, 2004; Michalski et al., 2004; Sturchio et al., 2009; Jackson et al., 2010). Regionally low NO$_3^-$ production does not seem likely as the overall average Cl$^-$/NO$_3^-$ molar ratio (1.1) is the fourth lowest of all locations and matches reasonably well with reported deposition ratios for the Atacama (0.74) (Ewing et al., 2006). An anomaly in the deposition flux of ClO$_4^-$ presumably would require a mechanism to localize atmospheric production, as other sites in the southern hemisphere do not have such low NO$_3^-$/ClO$_4^-$ ratios. Atacama ClO$_4^-$ has a unique isotopic composition compared to other reported natural ClO$_4^-$ from Mojave, Rio Grande Basin, and Southern High Plains (Böhlke et al., 2005; Jackson et al., 2010; Sturchio et al., 2011). The unique isotopic composition (low $\delta^{37}$Cl, $\delta^{18}$O, and $^{36}$Cl/Cl) could be related to an additional unknown production mechanism which could also explain the very low ratios of NO$_3^-$/ClO$_4^-$. Alternatively, given the duration of hyper-arid conditions in the Atacama (>2 million years), the relative enrichment and unique isotopic composition of ClO$_4^-$ could be a reflection of atmospheric conditions that are no longer present. The age of the ClO$_4^-$ in the Atacama has been estimated to be at least 750,000 years for the youngest samples, with a mean age of 3-8 million years based on $^{36}$Cl/Cl$^-$ ratios in ClO$_4^-$ (Sturchio et al., 2009). This suggests that almost all of the Atacama ClO$_4^-$ is older than 300,000 years, and thus predates the estimated time period over which ClO$_4^-$ was deposited at other terrestrial locations.

2.3.5.4 Implications of Cl$^-$/NO$_3^-$/ClO$_4^-$ Global Correlations

The global consistency of NO$_3^-$/ClO$_4^-$ ratios in all locations other than the Atacama is in contrast to the more variable Cl$^-$/NO$_3^-$ and Cl$^-$/ClO$_4^-$ ratios (Figure 2.3.7). Cl$^-$/NO$_3^-$ ratios are commonly used to evaluate N and/or NO$_3^-$ gains/losses in various ecosystems. Co-variation of Cl$^-$/NO$_3^-$ and Cl$^-$/ClO$_4^-$ ratios illustrated in Figure 2.3.7 indicates large variations in Cl$^-$ are related to processes that either (1) do not affect NO$_3^-$ or ClO$_4^-$, or (2) affect both NO$_3^-$ and ClO$_4^-$ similarly. Atmospheric deposition plots at the low end of the correlated data array in Figure 2.3.7, indicating that many soils, caliches, unsaturated salt bulges, and groundwaters in arid and semi-arid regions have NO$_3^-$/ClO$_4^-$ ratios similar to atmospheric deposition ratios, whereas Cl$^-$/NO$_3^-$ and Cl$^-$/ClO$_4^-$ ratios of terrestrial samples are systematically higher than deposition ratios. For the deposition data summarized in this paper (U.S. wet deposition (WD) and Mojave total deposition (TD)), the Cl$^-$/NO$_3^-$ molar ratios (0.33 and 0.61 for WD and TD, respectively) are similar to that previously estimated from oxic groundwater data for the Middle Rio Grande Basin (Cl$^-$/NO$_3^-$ = 0.27) (Plummer et al., 2006) as well as that calculated using reported data (2000-2010) for total deposition from 95 sites across the U.S. (Cl$^-$/NO$_3^-$ = 0.29; n=892) (CASTNET, 2014).

Cl$^-$ concentrations in atmospheric deposition can vary independently of the concentrations of constituents with atmospheric sources. For example, Rajagopalan et al. (2006) reported mean values of Cl$^-$/NO$_3^-$ and Cl$^-$/ClO$_4^-$ in wet deposition at different sites across the U.S. varied by 2
orders of magnitude and much of the variation was related to distance from the coast, presumably reflecting variation in the sea-salt component relative to other components of the Cl\(^{-}\) deposition. Similarly, continental-scale studies of \(^{36}\)Cl/Cl ratios in pre-anthropogenic groundwater in the U.S. and modern atmospheric deposition in Europe indicated maximum coastal marine Cl\(^{-}\) enrichment factors on the order of 20-40 (Davis et al., 2003; Johnston and McDermott, 2008). Varying deposition ratios could account for some of the regional terrestrial variation illustrated in Figure 2.3.7, but probably not all of it, and presumably would not cause almost all terrestrial samples to be relatively enriched in Cl\(^{-}\). The Cl\(^{-}\)/NO\(_3^{-}\) ratios of soil/caliche and groundwater samples vary over 3 orders of magnitude, with little relation to distance from the coast, although some of the highest Cl\(^{-}\)/NO\(_3^{-}\) ratios are from areas near coasts (e.g., Namibia and UAE). The highest wet deposition Cl\(^{-}\)/NO\(_3^{-}\) ratio observed by Rajagopalan et al., (2006) for 4 coastal sites (<50km) was 17 (Puerto Rico) while the other 3 coastal sites had ratios <4.

The array of data extending from atmospheric deposition toward higher Cl\(^{-}\)/NO\(_3^{-}\) and Cl\(^{-}\)/ClO\(_4^{-}\) ratios (Figure 2.3.7), and the lack of a relation between the increase in Cl\(^{-}\)/NO\(_3^{-}\) ratio and the NO\(_3^{-}\)/ClO\(_4^{-}\) ratio (Figure 2.3.7) suggests that either Cl\(^{-}\) at most sites has been supplemented by sources unrelated to the source of the original co-deposited NO\(_3^{-}\) or ClO\(_4^{-}\), or that both NO\(_3^{-}\) and ClO\(_4^{-}\) were equally lost in these systems as previously described. Partial microbial reduction of NO\(_3^{-}\) is supported by isotope data at some sites (Figure 2.3.6). However, NO\(_3^{-}\) isotopes at most sites appear to be relatively unfractionated and some indicate substantial unaltered atmospheric NO\(_3^{-}\). For ClO\(_4^{-}\) and NO\(_3^{-}\) to be lost due to episodic reduction without apparent isotope effect would require complete loss at micro-sites and as much as 99% total loss to account for the magnitude of change in the Cl\(^{-}\)/NO\(_3^{-}\) ratios, which is considered improbable given the current aridity of most locations in this study. However, as previously mentioned, it is possible that some sites with elevated Cl\(^{-}\) concentrations contain atmospheric Cl\(^{-}\) that accumulated during previous wetter periods that supported biological reduction of NO\(_3^{-}\) and ClO\(_4^{-}\) (non-co-deposited Cl\(^{-}\)).

Another possible confounding factor may be the potential de-coupling of Cl\(^{-}\) and NO\(_3^{-}\) or ClO\(_4^{-}\) transport. Local redistribution of salts by selective crystallization, dissolution, and infiltration might have separated NO\(_3^{-}\) and ClO\(_4^{-}\) from Cl\(^{-}\) at the scale of individual samples or vertical profiles. Also, we have observed that at least some plants uptake ClO\(_4^{-}\) and NO\(_3^{-}\) proportionally while excluding Cl\(^{-}\) (Andraski et al., 2014). Biologic assimilation could be responsible for substantial N sequestration in persistent organic matter, and release of N gases (e.g., NH\(_4\), N\(_2\)O, N\(_2\)) during remineralization, nitrification, or denitrification, could also lead to elevated Cl\(^{-}\)/NO\(_3^{-}\) ratios in soils and recharging groundwaters, but existing data do not support similar losses of ClO\(_4^{-}\) due to plant uptake (Andraski et al., 2014, Tan et al., 2004b, 2006). It is emphasized that the Atacama Desert occupies a unique position in all of the figures that present data from this study, which can only be achieved by assuming a localized in-situ production mechanism or
unknown variation in the deposition ratios over million-year time scales that would not be 
recorded at the other sites.

2.3.6 Conclusions

ClO$_4^-$ is globally distributed in soil and groundwater in arid and semi-arid regions on Earth at concentrations ranging from $10^{-1}$ to $10^6$ µg/kg. Generally, the ClO$_4^-$ concentration in these regions increases with aridity index, but also depends on the duration of arid conditions. In many arid and semi-arid areas, NO$_3^-$ and ClO$_4^-$ co-occur at consistent ratios (NO$_3^-$/ClO$_4^-$) that vary between $\sim$10$^4$ and $\sim$10$^5$. These ratios are largely preserved in hyper-arid areas that support little or no biological activity (e.g. plants or bacteria), but can be altered in areas with more active biological processes including N$_2$ fixation, N mineralization, nitrification, denitrification, and microbial ClO$_4^-$ reduction. At first approximation, relatively constant NO$_3^-$/ClO$_4^-$ ratios in desert environments are consistent with global atmospheric sources, and with dry deposition as a major controlling factor.

In contrast, much larger ranges of Cl$^-$/ClO$_4^-$ and Cl$^-$/NO$_3^-$ ratios indicate Cl$^-$ varies independently from both ClO$_4^-$ and NO$_3^-$. This is likely due to variation of sea salt Cl$^-$ in deposition, plus variable addition of Cl$^-$ from dust or subsurface sedimentary and evaporitic sources, and possibly excess Cl$^-$ accumulation during past wetter periods of NO$_3^-$ and ClO$_4^-$ reduction. The general lack of correlation between Cl$^-$ and ClO$_4^-$ or NO$_3^-$ implies that Cl$^-$ is not a good indicator of co-deposition and should be used with care when interpreting oxy-anion cycling and mass balances in arid systems.

In general, the co-occurrence of ClO$_4^-$ and NO$_3^-$ in arid and semi-arid locations, and associated variations in the isotopic composition of the NO$_3^-$, are consistent with a conceptual model of atmospheric origin, global co-deposition, and variable alteration of the NO$_3^-$ pool by biogenic addition, assimilation, and/or recycling on the surface. Preservation of the atmospheric deposition NO$_3^-$/ClO$_4^-$ ratio in unsaturated-zone accumulations and groundwater in areas where biological activity is substantial and on-going appears to indicate limits to net gains and losses of N through biological processes in soils between deposition inputs and infiltration outputs.

Measurements of ClO$_4^-$ concentration in desert regions could be useful for interpreting major anion cycles and processes, particularly with respect to the less-conserved NO$_3^-$ pool. If more accurate local measures of deposition can be obtained, then ClO$_4^-$ surface accumulations in conjunction with NO$_3^-$ isotopic composition could be useful in evaluating the duration of arid conditions that led to such accumulations, and potentially also the extent of biological processes and nitrogen mass transfers.

The Atacama Desert appears to be unique compared to other arid and semi-arid locations. There, exceptional enrichment in ClO$_4^-$ compared to Cl$^-$ or NO$_3^-$, accompanied by unique ClO$_4^-$ isotopic
characteristics (Bao and Gu, 2004; Böhlke et al., 2005; Jackson et al., 2010), may reflect an unusually efficient, but yet unknown, *in situ* production mechanism, regionally elevated atmospheric ClO₄⁻ production rates, or higher ClO₄⁻ production rates in pre-Pleistocene times.

In the absence of microbial reduction, ClO₄⁻ can persist in many environmental conditions. On early Earth, prior to the onset of microbial ClO₄⁻ reduction, ClO₄⁻ concentrations could have been much higher than currently observed. However, the net impact of microbial ClO₄⁻ reduction on the global chlorine biogeochemical cycle remains unconstrained.

Elevated concentrations of ClO₄⁻ reported on the surface of Mars, and its enrichment with respect to Cl⁻ and NO₃⁻, could reveal important clues regarding the climatic, hydrologic, and potentially biologic evolution of the planet. Given the highly conserved ratio of NO₃⁻/ClO₄⁻ in non-biologically active areas on Earth, it may be possible to use alterations of this ratio as a biomarker on Mars.
2.4 Perchlorate and Chlorate in the Ice-Covered Lakes of McMurdo Dry Valleys, Antarctica.

2.4.1 Background

In section 2.3, we summarized data concerning ClO$_4^-$ concentrations in Antarctica soils in conjunction with a worldwide overview of natural anion concentrations and relationships in soils and groundwater. In this section, we provide the data concerning ClO$_4^-$ and ClO$_3^-$ in ice-covered lakes in Antarctica.

Ice-covered lakes are the main sites for biological activity in the McMurdo Dry Valleys (MDV) of Antarctica, and represent one of the most pristine aquatic ecosystems on Earth. These lakes have been extensively studied in relation to their physical structure, ecology, and biogeochemistry (e.g. Green and Lyons, 2009 and references therein). Most of the ice-covered lakes in the MDV contain both oxic and anoxic zones within the water column, although several lakes are oxic throughout. Lake water temperatures are always close to 0ºC, and the water columns are characterized by relatively low biological productivity. In all cases, the lakes are perennially ice-covered, which influences all physical, chemical, and biological processes. The deep waters of the east lobe of Lake Bonney rank among the most saline aqueous environments on our planet with very limited or no microbial activity, while other lakes contain essentially fresh water and support diverse and widespread photosynthetic microbial mats, as well as chemolithotrophic and heterotrophic processes such as nitrification, sulfur oxidation, denitrification, and sulfate reduction (Voytek et al., 1999, Green and Lyons, 2009).

Many major and trace element geochemical and biogeochemical processes have been evaluated in these lakes (e.g., Lee et al., 2004), but no information is available concerning the occurrence, distribution and fate of ClO$_3^-$ and ClO$_4^-$. The purpose of this task was to measure ClO$_4^-$ and ClO$_3^-$ levels in ice-covered lakes of the MDV, and to evaluate whether these species were being reduced by bacteria capable of (per)chlorate reduction. Results are discussed with regard to the ecology, chemical evolution, and history of lakes in the MDV.

2.4.2 Materials and Methods

ClO$_4^-$, ClO$_3^-$, NO$_3^-$, SO$_4^{2-}$, and Cl$^-$ concentrations were measured for samples from a number of surface water bodies (e.g. streams, creeks, ponds; see Table 2.4.1) and 4 ice-covered lakes within the MDV: Lake Bonney (East and West Lobe), Lake Hoare, and Lake Fryxell in Taylor Valley and Lake Miers in the Miers Valley. All samples were obtained from the Long Term Ecological Research group (LTER) using techniques previously outlined (http://www.ternet.edu/sites/mcm/). Samples were collected in either December of 2008 (Lake Fryxell and Miers) or 2009 (Lake Hoare and Bonney). Samples were filtered (GF/F filter) within 6-8 hours of collection and stored frozen.
2.4.3 Analysis

Major anions (Cl\(^-\), NO\(_3^+\), and SO\(_4^{2-}\)) were analyzed by ion chromatography as described previously in this report. Anion concentration distribution within the lakes is well documented but was evaluated here to demonstrate consistency with past efforts and to evaluate ClO\(_4^+\) and ClO\(_3^-\) concentrations with respect to other anions on a consistent set of samples. ClO\(_4^+\) and ClO\(_3^-\) concentrations were separately measured by IC-MS/MS as previously described in this report following the method detailed in Rao et al., (2007 and 2010b). The MDL was 2 ng/L for both ClO\(_3^-\) and ClO\(_4^+\), while the MRL for these samples was 50 ng/L. Samples were analyzed in batches of 8 including an analytical duplicate and spike. The errors in duplicate samples were less than ± 10 % and the spike recoveries were between 90 and 110 %. Samples with elevated Cl\(^-\) (>10,000 mg/L) or SO\(_4^{2-}\) (>1000 mg/L) were either diluted prior to analysis or in some cases pre-cleaned using On-Guard\textsuperscript{TM} II Ag or Ba cartridges (Dionex).

2.4.4 Modeling

Two simple models were used to evaluate ClO\(_4^+\), ClO\(_3^-\), and SO\(_4^{2-}\) concentrations relative to the conserved species Cl\(^-\) in order to establish if non-transport related processes (e.g. bacterial reduction and evapoconcentration) were affecting the distribution of these species with depth in the water column. For Lakes Hoare, Miers, and Fryxell, for which the source of salts in the lakes is glacier and snow melt augmented by chemical weathering and possibly input of relic seawater for the hypolimnion of Lake Fryxell (Lyons et al., 1998 and 2000), we assumed that in the absence of sinks all anions should increase in concentration proportional to Cl\(^-\). For each species we calculated the mass ratio with respect to Cl\(^-\) (e.g. ClO\(_4^+/\text{Cl}^-\)) at the depth closest to the bottom of the lake ice. This ratio was then multiplied by the Cl\(^-\) concentration at lower depths to determine the expected concentration of each species as shown below.

\[
C_{Dx} = \frac{C_{Ds}}{Cl_{Ds}} \times Cl_{Dx}
\]

Where \(C_{Dx}\) and \(C_{Ds}\) are the concentration of ClO\(_4^+\), ClO\(_3^-\), or SO\(_4^{2-}\) at depth X and the surface, respectively; and \(Cl_{Dx}\) and \(Cl_{Ds}\) are the concentrations of Cl\(^-\) at depth X and the surface, respectively. For Lake Bonney, for which the salts are attributed to both surface inflow and relic seawater (Lyons et al., 2005), we used a simple two component mixing model. We calculated the mixing ratio of surface and water from 35m (deepest depth) to produce the observed Cl\(^-\) profile using a simple mass balance

\[
Cl_{Dx} = f_{Dx} Cl_{Ds} + f_{35} Cl_{35}
\]

where \(f_{Dx}\) and \(f_{35}\) are the fraction of surface water and water at 35m respectively at any depth \(D_x\), \(Cl_{35}\) is the concentration at 35m and all other variables are previously defined. The values of \(f_{Dx}\) and \(f_{35}\) determined for each depth using Cl\(^-\) were then used to calculate the concentration of
ClO₄⁻, ClO₃⁻, or SO₄²⁻ using the same mass balance but substituting the concentrations of each species for Cl⁻ at the surface and at 35m to predict the concentration at intermediate depths. Modeled results are only used to highlight processes impacting oxyanions relative to Cl⁻. The modeled concentrations are plotted as dashed lines on the figures presenting the concentration distribution with depth for each Lake.

2.4.5 Results and Discussion

2.4.5.1 Surface Waters in Wright and Taylor Valley

The permanently ice-covered lakes in the MDV are supplied by glacial melt and include streams, creeks, and the Onyx River that feed various lakes as well as ponds (Figure 2.4.1). ClO₄⁻ concentrations in a subset of these water bodies ranged from 0.05-8.1 μg/l but were generally less than 0.5 (45/49 samples) with an overall average of 0.19 μg/l excluding one outlier (Parera Pond) (Table 1). Of the samples that exceeded 0.5 μg/l all but one (Parera Pond) were in streams that feed Fryxell Lake. Only two samples (Green Creek and Blood Falls) were below the detection limit (0.05μg/l). In most cases, we report concentrations for multiple samples from the same water body, taken at either different dates and/or different locations. In these instances, concentrations are generally within a factor of 2 of each other. Most ClO₃⁻ and ClO₄⁻ concentrations in surface waters of the MDV are higher than those reported for wet precipitation from North America (average = 0.014 μg/L) (Rajagopalan et al., 2009) or in ice cores from Wyoming (0.0002< 0.009 μg/L), Yukon Territory (0.0002 < - 0.002 μg/L), and the Devon Ice Cap (0.001-0.015 μg/L) (Rao et al., 2012; Furdui and Tomassini, 2010). The higher concentrations in the MDV surface water are not surprising given that they include both dry and wet deposition from glacier melt, which may have experienced evapo-concentration due to ice sublimation as well as input from dissolution of salts from the surrounding catchment. ClO₃⁻ concentrations in the non-lake surface water bodies were similar to ClO₄⁻ with concentrations ranging from <0.05 to 15.5 μg/l and an overall average excluding Goldman Pond of 0.25 μg/l (Table 2.4.1). Little information is available concerning ClO₃⁻ in the environment but ratios of ClO₃⁻/ClO₄⁻ in North American precipitation and evaporites from the Mojave, Atacama and Namibia Deserts generally plot close to a 1:1 (W/W) ratio line (Rao et al., 2010b) and ice core samples from the Devon Island Ice Cap have an average ratio (W/W) of 3.8 ±2.9 (Furdui and Tomassini, 2010). Samples from Taylor and Wright Valleys have an average ratio (W/W) of 1.7±1.1 similar to other reported samples.

2.4.5.2 Ice-covered lakes in Taylor and Miers Valley

Lake Hoare

Lake Hoare is a fresh water lake ponded by the Canada Glacier (Figure 2.4.1). The lake water is relatively young ~1000-2500 yr and the source of Cl⁻ and other salts is attributed to snow and glacial melt, as well as weathering of minerals and dissolution of dust on melting glaciers (Lyons et al., 1998; Lyons et al., 2005; Voytek et al., 1999). The lake is uniformly oxic (~20 mg O₂/l) to
a depth of ~17m at which point oxygen sharply decreases to near 0 at the sediment interface (Clocksin et al., 2007). There is a chlorophyll-a peak at 15m and NO$_3^-$ is below detection (<0.5 µg N/l) above 15m and increases due to nitrification below 15m reaching a maximum of ~112 µgN/l (Voytek et al., 1999). Microbial analysis indicates that nitrifiers are present at all depths and of the eight isolates obtained all were obligate aerobes (Clocksin et al., 2007 and Voytek et al., 1999). The presence (~0.7 mg/l) of H$_2$S near the sediment-water interface clearly indicates anoxic processes are occurring near the sediment water interface (Clocksin et al., 2007).

Our results show that ClO$_4^-$ and ClO$_3^-$ in Lake Hoare are present throughout the water column at concentrations ranging from 0.1 to 0.3 and 0.15 to 0.42 µg/l, respectively (Figure 2.4.2). Concentrations were lowest near the surface and at the sediment interface with a peak concentration near 10m. As previously reported, Cl$^-$ concentrations decrease exponentially towards the surface. SO$_4^{2-}$ follows a similar trend increasing proportionally to Cl$^-$ with the exception of the deepest sample (30m) consistent with the presence of H$_2$S at the same depth. Neither ClO$_4^-$ nor ClO$_3^-$ concentrations increase proportionally to Cl$^-$, although the concentrations do initially increase.

This relationship with Cl$^-$ is further highlighted by the predicted concentrations (dashed lines Figure 2.4.2) of these species using a simple model based on maintaining the initial ratios of each species relative to Cl$^-$ in the lake surface water with depth. The ratios of SO$_4^{2-}$ to Cl$^-$ are largely conserved with depth except as discussed near the sediment water interface. SO$_4^{2-}$ reduction has apparently not been extensive enough to impact the concentration of SO$_4^{2-}$ in the majority of the lake profile (Figure 2.4.2). The ratio of ClO$_4^-$ and ClO$_3^-$ to Cl$^-$ in the lake surface water are close to the measured values in stream input to Lake Hoare (Figure 2.4.3). Predicted concentrations of ClO$_3^-$ and ClO$_4^-$ based on Cl$^-$ concentration with depth are up to an order of magnitude higher than measured concentrations indicating a depletion of ClO$_x^-$ for all depths below the surface water.

The concentration of ClO$_4^-$ and ClO$_3^-$ can likely be explained by a similar process as SO$_4^{2-}$. Like NO$_3^-$ both ClO$_4^-$ and ClO$_3^-$ are only reduced at oxygen concentrations below ~1 mg/l. Therefore given the elevated O$_2$ concentrations throughout the lake depth, ClO$_4^-$ and ClO$_3^-$ are unlikely to be reduced in the bulk lake water. However, as sulfate reduction is clearly occurring in the lake sediment the conditions exist to support ClO$_x^-$ reduction. Both ClO$_4^-$ and ClO$_3^-$ are utilized as electron acceptors prior to SO$_4^{2-}$, and consequently the upper portions of the sediment should support ClO$_x^-$ reduction. The very large differences in concentrations of SO$_4^{2-}$ and ClO$_x^-$ (SO$_4^{2-}$/ ClO$_x^-$ >10,000) explain the difference in ClO$_x^-$ depth profiles compared to SO$_4^{2-}$. The relatively low organic matter input to the sediments sustains relatively low rates of electron acceptor (O$_2$, NO$_3^-$, SO$_4^{2-}$) reduction, but given the ratio of SO$_4^{2-}$ to ClO$_x^-$ this rate would lead to the rapid consumption of ClO$_x^-$ but a negligible decrease in SO$_4^{2-}$ concentration. This would eventually
lead to a depleted ClO$_x^-$ concentration profile relative to Cl$^-$ throughout most of the depth of the lake as observed. Hence, one implication of the data presented here is that ClO$_x$ species can be used as a very sensitive marker for microbial activity in the water column of ice-covered lakes. Finally, the presence of trace amounts of NO$_3^-$ at lower depths suggests that it is being produced in excess of the consumption rate.

**East Lobe Lake Bonney**

Lake Bonney is the largest lake in Taylor Valley with a maximum depth of ~40m (Green and Lyons, 2009). It has two lobes (west and east) that have had very different evolutionary histories (Matsubaya et al., 1972; Poreda et al., 2004). In the East Lobe (EL), the surface water is fresh (~0.6g TDS/l) to highly saline at depth (~273 g TDS/l). Both lobes are vertically stabilized by the strong salt gradients in the lake (Spigel and Priscu, 1998). The lake has existed in some form for a minimum of 300,000 years and the hypolimnion has been attributed to a Tertiary period marine fjord (Hendy, 2000; Lyons et al., 2005). The EL geochemistry has been modified due to the loss of ice cover in the mid-Holocene which reformed ~200 years ago. The loss of the ice cover led to cryo-concentration causing precipitation of various salts leading to a thick deposit of NaCl on the surface sediments. The lake geochemistry has been further modified by input from the west lobe due to overflow and input from glacier inflow including weathering products. Dissolved Organic Carbon (DOC) increases with depth reaching a maximum of 30 mg/l and is attributed to primary production in the lake, evapoconcentration (Green and Lyons, 2009) and subglacial flow from the Taylor Glacier (Mikucki et al. 2004).

The biogeochemistry of the EL is to some extent not fully understood. Oxygen is above saturation (~20mg/l) above 20m and rapidly declines to suboxic concentrations (<1 mg/l) across the chemocline (20-23m). Dissolved inorganic nitrogen (NO$_3^-$, NO$_2^-$, NH$_4^+$) rapidly increases below the chemocline reaching stable maximums (2.4, 0.6, and 3.7 mg-N/l, respectively) at a depth ~30m and below (Priscu et al., 2008). Dissolved N$_2$O in EL increases similarly below the chemocline and declines rapidly (<1uM) below 30m. The anomalously elevated concentrations of NO$_3^-$ and NO$_2^-$ under suboxic conditions in the deep water are not understood, but multiple studies indicate that denitrification is either not occurring or is occurring at such low rates as to have minimal impact (Priscu et al., 2006, 2008; Ward and Priscu, 1997). Factors contributing to the lack of denitrification activity include the elevated TDS, low temperatures, and elevated redox potential (>400mV) (Ward et al., 2005; Ward and Priscu, 1997). Isotopic studies of $^{15}$N and $^{18}$O in N$_2$O in concert with genomic studies suggest the origin of the N$_2$O was due to nitrification and the current profiles are attributed to a legacy of a former biogeochemical condition that have been preserved due to the extreme stability of the lake (Priscu et al., 2008). Sulfate concentrations increase with depth but the ratio of SO$_4^{2-}$/Cl$^-$ decreases from the surface to the top of the chemocline (~20m) below which it remains reasonably constant. Given the elevated redox condition, the presence of NO$_3^-$, NO$_2^-$, as well as the lack of any H$_2$S, the
The reduction in ratio is most likely due to precipitation of a SO$_4^{2-}$ mineral phase in the hypersaline waters rather than sulfate reduction.

ClO$_4^-$ concentration increases with depth in the EL of Lake Bonney (0.46 to 8.3 ug/l at the surface and 35m, respectively) (Figure 2.4.4). The increase in ClO$_4^-$ concentration is proportional to the increase in Cl$^-$ concentration ($r^2=0.99$) over the entire water column. ClO$_4^-$ and SO$_4^{2-}$ concentrations are predicted accurately with a simple mixing model of concentrations at the surface and 35m (Dashed lines Figure 2.4.4). The ClO$_4^-$/Cl$^-$ molar ratio (5X10$^{-7}$) at the shallowest depth (4m) is less than the ratio in Taylor Valley surface streams (4X10$^{-6}$) and decreases with depth to a relatively constant value of ~6 X10$^9$ at 35m (Figure 2.4.3). The ratio at 35 m is still substantially enriched compared to seawater 3.0 X10$^{-10}$ which is considered the major source of salts in the EL. It is possible that water below 18m became enriched in ClO$_4^-$ relative to Cl$^-$ from in situ precipitation of NaCl which is reported to be at least 1.6m and up to 10m thick in EL sediments (Hendy et al., 1977). Cl$^-$ concentrations reach a maximum at 24m and remain constant at lower depths (Lyons et al., 2005). ClO$_4^-$ would not precipitate given its low concentrations relative to other anions and its very high solubility. There is no evidence (such as a selective loss of ClO$_4^-$ at depth with respect to Cl$^-$) of biological reduction of ClO$_4^-$ which is not unexpected given the stability of NO$_3^-$ and NO$_2^-$ at depth and the noted lack of any significant nitrate reduction (Priscu et al., 2008, Ward et al., 2005, Ward and Priscu, 1997). All of this suggests the ClO$_4^-$ concentration profiles and ClO$_4^-$/Cl$^-$ ratio profiles are a product of dilution of evapoconcentrated seawater by surface inflow and subsequent diffusion which is consistent with the proposed history of the EL. The origin of ClO$_4^-$ in the EL could be a mix of surface water inflow and seawater, although it is currently not possible to determine if the ClO$_4^-$ in the original seawater is still present. It is possible the east lobe could have experienced conditions that supported ClO$_4^-$ reduction in the distant past given the estimated age of isolation of the seawater 1.7-5.1Ma. Regardless, the ClO$_4^-$ must be reasonably old as the concentration at 35 m is at least ~70X the average Taylor Valley stream concentration and 170X seawater concentrations.
**Table 2.4.1. ClO₃⁻, ClO₄⁻, Cl⁻ and SO₄²⁻ concentrations in surface waters of the Dry Valleys.**

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<th>ClO₃⁻</th>
<th>ClO₄⁻</th>
<th>Cl⁻</th>
<th>SO₄²⁻</th>
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</tbody>
</table>

*Common Wealth stream does not feed Lake Fryxell but Common Wealth Glacier, its source, does provide inflow to Lake Fryxell and so it was grouped in this basin.*
Figure 2.4.1. Map of Taylor Valley, Antarctica showing the locations of Lake Fryxell, Lake Bonney, and Lake Hoare. Adapted from Lyons et al., (2005).
Figure 2.4.2. Concentration profiles of ClO$_4^-$ and ClO$_3^-$, Cl$^-$ and SO$_4^{2-}$ in Lake Hoare, December 2009. Dashed lines represent modeled concentrations based on maintaining surface water ratios of ClO$_3^-$, ClO$_4^-$ and SO$_4^{2-}$ relative to Cl$^-$ throughout the lake depth.
Figure 2.4.3. Molar ratios of ClO$_4^-$/Cl$^-$ and ClO$_3^-$/Cl$^-$ with depth in MCM lakes and feed waters for lake basins in Taylor Valley and the Onyx River in Wright Valley. The black dashed line in each figure represents the ratio in seawater sampled at McMurdo Station.
In contrast to ClO₄⁻, ClO₃⁻ is not as conservative with respect to Cl⁻ concentration. ClO₃⁻ concentration does increase with depth (1.1 and 10.5 ug/l at 4m and 35m, respectively) but the increase is proportional to Cl⁻ only to a depth of 18m (Figure 2.4.4). This is highlighted by evaluating the relationship between ClO₃⁻ and ClO₄⁻ (Figure 2.4.5) which is highly correlated (R=0.97) up to a depth of 18m. The predicted ClO₃⁻/ClO₄⁻ ratio (3.1) is very similar to the ratio in Taylor Valley surface streams (2.2). However, the sample point at 35m is clearly depleted in ClO₃⁻ relative to ClO₄⁻. Modeled ClO₃⁻ concentrations at 4, 12, and 18m are well predicted based on the two parts mixing model and using the predicted ClO₃⁻ concentration at 35m based on the ClO₄⁻ concentration. ClO₃⁻/Cl⁻ molar ratios in EL surface water (1X10⁻⁶) are similar to streams throughout Taylor Valley and are still well above seawater ratios (1X10⁻¹⁰) even with the apparent reduction of ClO₃⁻ at 35m. What is clear is that at least at 35m some loss of ClO₃⁻ relative to ClO₄⁻ has occurred. This could be due to low level ClO₃⁻ reduction by low level nitrate reductase activity. While NO₃⁻ and NO₂⁻ concentrations appear to be temporally stable at 35m and denitrification is reported to be either absent or at most occurring at insignificant rates (Priscu et al., 2008; Ward et al., 2005; Ward and Priscu, 1997), it is still possible that ClO₃⁻ has been reduced. ClO₃⁻ concentrations are only 0.1 and 0.3% of the molar concentrations of NO₃⁻ and NO₂⁻, respectively and therefore reduction rates too small to impact bulk NO₃⁻ and NO₂⁻ could have an impact on ClO₃⁻ concentrations over long periods. Whether this reduction is ongoing or simply a fossil of previous conditions in the EL is unknown.

West Lobe Lake Bonney
The West Lobe (WL) is much smaller than the EL and exchanges water with the EL down to 13m at which depth a sill separates the deeper waters of the east and west lobes. The water quality above the sill layer is therefore similar between the two lobes but the deeper depths are distinct presumably due to their separate evolutionary histories. The WL has apparently not ever lost its ice cover (Hendy et al., 1977; Matsubaya et al., 1979). The source of the deeper water is also attributed to seawater which has been cryo-concentrated but to a lesser extent (144 g/l) due to the presence of the ice cover (Lyons et al., 2005), and from subglacial outflow from the Taylor Glacier (Mikucki et al. 2004 and 2009). A number of lines of evidence suggest that the water may be quite old. The biogeochemistry of the WL follows a more conventional redox profile (Lee et al. 2004). Oxygen concentrations are supersaturated above 13m and decline rapidly to less than 1 mg/l at ~25m. NO₃⁻, NO₂⁻, and N₂O all peak in or near the chemocline (15-18m) and then rapidly decline below the oxycline due to denitrification (Voytek et al., 1999; Priscu et al., 1996; Ward and Priscu, 1997). Ammonium and DOC concentrations steadily increase below the chemocline but hydrogen sulfide is not present (Green and Lyons, 2009; Voytek et al., 1999; Downes et al., 1995).

Concentration profiles of ClO₄⁻ and ClO₃⁻ are similar to the EL except at the lowest sampling depth (35m) at which concentrations are ~5 and 8X lower even though Cl⁻ is only 2X lower.
(Figure 2.4.6). ClO$_4^-$/Cl$^-$ and ClO$_3^-$/Cl$^-$ ratios are also similar for comparable depths (Figure 2.4.3). The loss of ClO$_3^-$ relative to ClO$_4^-$ is also similar to the EL (Figure 2.4.5). Predicted concentrations based on the two component mixing model are reasonably similar to measured values for SO$_4^{2-}$ and ClO$_4^-$, although some loss of ClO$_4^-$ has occurred at 35m. Predicted concentrations of ClO$_3^-$ are similar for depths above 35m if the concentration at 35m is predicted from the ClO$_4^-$ concentration and expected ratio of ClO$_3^-$/ClO$_4^-$ from Figure 2.4.5. ClO$_4^-$ concentration at 35m and the ratio from Figure 2.4.5 is used to predict the ClO$_3^-$ concentration at 35m rather than the measured concentration. The differences between the EL and WL are likely due to the biological reduction of ClO$_4^-$, ClO$_3^-$ and NO$_3^-$ at depth in the WL as opposed to only ClO$_3^-$ in the EL. The WL has an active denitrification zone below 18m and NO$_3^-$, NO$_2^-$ and N$_2$O are completely reduced by 30m (Voytek et al., 1999; Priscu et al., 1996, Priscu 1997). Taken together this implies that loss of ClO$_4^-$ and ClO$_3^-$ was active in the past but has now ceased or that loss commenced in the relatively near past compared to the age of the lake. Otherwise, we would expect much lower concentrations of both species at depth especially with respect to ClO$_4^-$ which is only slightly depleted with respect to Cl$^-$.

**Lake Fryxell**
The geochemistry of Lake Fryxell has recently been reviewed by Green and Lyons (2009). TDS varies from near fresh water at the surface (0.99g/l) to brackish (7.8g/l) water at depths below the oxicline (~9.5m) where high levels of hydrogen sulfide exist. The lake has been subject to a number of draw down and refill events (Wagner et al., 2006). The conserved Cl$^-$ profile steadily increases with depth and has previously been attributed to diffusion of Cl$^-$ from the sediment to the surface (Green and Lyons, 2009). At depths above ~9m the water is supersaturated with O$_2$ and between ~9-11m a chemocline exists in which O$_2$ is rapidly depleted and in which chlorophyll-a reaches a concentration maximum (Priscu, 1995). SO$_4^{2-}$ slowly increases with depth to a maximum at ~11-12m and then rapidly decreases to below 0.1mM at the sediment water interface. H$_2$S reaches a maximum (1.4mM) at the sediment water interface and declines steadily to 9m above which it is not present (Karr et al., 2005; Sattley and Madigan, 2006; Aiken et al., 1996). An active sulfur oxidizing population is present with a population maximum within the chemocline but extending into the anoxic depths. DOC distribution is similar to Cl$^-$ with a minimum at the surface (~3.0 mg/l) and a maximum (31.2 mg/l) at the sediment water interface. Like Cl$^-$ the distribution of DOC is attributed to diffusion from the sediment of degraded and relic sediment organic matter, and new input from surface water inflow (Aiken et al., 1996). NH$_4^+$ was reported to be near the detection limit from the surface to the bottom of the chemocline, below which the concentration steadily increases to a maximum (5.6 mg-N/l) at the sediment water interface. NO$_3^-$ was below detection for all depths and NO$_2^-$ was present at trace concentrations (<0.0042 mg-N/L) at 7m and below 15m (Voytek et al., 1999).
Concentrations of both ClO$_4^-$ and ClO$_3^-$ increase slightly from 5m to a maximum at 7m and then decline steadily to just below the chemocline at 10m below which they remain relatively constant (Figure 2.4.7). Concentrations at depths above 9m are generally similar to surface water concentrations in Taylor Valley (Table 2.4.1). The ratio of ClO$_4^-$/Cl and ClO$_3^-$/Cl are the lowest of all the lakes, likely due to both the addition of Cl$^-$ from the sediment and strongly reducing conditions below 11m that facilitate ClO$_4^-$ and ClO$_3^-$ bacterial reduction. Predicted concentrations based on surface water ratios of ClO$_4^-$/Cl$^-$ and ClO$_3^-$/Cl$^-$ over estimate both ClO$_4^-$ and ClO$_3^-$ concentrations at depths below 6m. SO$_4^{2-}$ concentrations are likewise over predicted at depths below the chemocline. Collectively, these results in concert with the complete lack of NO$_3^-$ and NO$_2^-$ below the chemocline (Priscu and Ward, 1999, Priscu 1997) suggests that ClO$_4^-$ and ClO$_3^-$ are actively being reduced. These data further imply that ClO$_4^-$ and ClO$_3^-$ were either not present during the last lake drawdown (~1000Ka) or were consumed since the lake has been refilled. The ClO$_3^-$ and ClO$_4^-$ concentration profile appear to reflect a surface source, distribution by diffusion, and consumption at depth below 11m.
Figure 2.4.4. Concentration profiles of ClO$_4^-$ and ClO$_3^-$, Cl$^-$ and SO$_4^{2-}$ in the EL of Lake Bonney, December 2009. Dashed lines represent modeled concentrations based on a two part mixing model of concentrations at the surface and 35m except for ClO$_3^-$ for which the concentration at 35m is predicted from the ClO$_4^-$ concentration using the ratio from Figure 2.4.5. See text for further explanation.
Figure 2.4.5. Relationship of ClO$_3^-$ and ClO$_4^-$ concentration in Dry Valley Lakes. The solid black line represents the regression line ($r^2 = 0.97$ and ratio $= 3.1$) and the red dashed line represents ratio of ClO$_3^-$ and ClO$_4^-$ in streams of Taylor Valley.
Figure 2.4.6. Concentration profiles of ClO$_4^-$ and ClO$_3^-$, Cl$^-$ and SO$_4^{2-}$ in the WL of Lake Bonney, December 2009. Dashed lines represent modeled concentrations based on a two part mixing model of concentrations at the surface and 35m except for ClO$_3^-$ for which the concentration at 35m is predicted from the ClO$_4^-$ concentration using the ratio from Figure 2.4.5. See text for more explanation.
Figure 2.4.7. Concentration profiles of ClO₄⁻ and ClO₃⁻, Cl⁻ and SO₄²⁻ in Lake Fryxell, December 2008. Dashed lines represent modeled concentrations based on maintaining surface water ratios of ClO₃⁻, ClO₄⁻ and SO₄²⁻ relative to Cl⁻ throughout the lake depth.
Lake Miers

Lake Miers is relatively unstudied compared to other MDV lakes. It is much younger (<300 years) and is the only lake with both hydraulic inflow and outflow and, as such, its chemistry resembles glacier meltwater. The lake is oxic throughout its depth but approaches anoxic conditions at the sediment water interface. Nitrification rates while not directly measured are believed to be very low and NO_3^- and NO_2^- are essentially constant with depth (<0.03 and 0.002 mg-N/l, respectively) (Voytek et al., 1999). ClO_3^- and ClO_4^- concentrations are essentially constant with depth with exception of depths below 14m where ClO_4^- concentrations decrease (Figure 2.4.8). Chloride increases with depth until 12m below the surface beyond which it remains relatively constant. ClO_3^-/Cl^- and ClO_4^-/Cl^- ratios in surface water are near but above Taylor Valley surface water and consistently decrease with depth (Figure 2.4.4). While the lake is oxic, the reduction in SO_4^{2-} below 14m strongly suggests that the sediments may support anoxic processes. This would be congruent with the reduction in ClO_4^-/Cl^- and ClO_3^-/Cl^- ratios.

Figure 2.4.8. Concentration profiles of ClO_4^- and ClO_3^-, Cl^- and SO_4^{2-} in Lake Miers, December 2008. Dashed lines represent modeled concentrations based on maintaining surface water ratios of ClO_3^-, ClO_4^- and SO_4^{2-} relative to Cl^-.
2.4.6 Summary and Conclusions

ClO$_3^-$ and ClO$_4^-$ are present throughout the water columns of the MDV lakes and other surface water bodies in the area. Concentrations among surface water bodies are generally similar reflecting a common atmospheric source. Variations in streams are likely due to site-specific processes, such as the degree of evaporative concentration and the differential input of salts due to leaching of soils and aeolian materials and subsequent inflow to streams. The concentrations of ClO$_3^-$ and ClO$_4^-$ in the ice-covered lakes are dependent on both the total evaporative concentration that has occurred as well as the biological activity within each lake. The two relatively young lakes (Miers and Hoare), have ClO$_3^-$ and ClO$_4^-$ concentrations and ratios of ClO$_3^-$/Cl$^-$ and ClO$_4^-$/Cl$^-$ in surface waters that are similar to source streams, but suggest ClO$_3^-$ and ClO$_4^-$ reduction at depth or in the sediments. Lake Fryxell has ClO$_3^-$ and ClO$_4^-$ concentrations similar to input streams but ClO$_3^-$/Cl$^-$ and ClO$_4^-$/Cl$^-$ ratios much lower due to the large Cl$^-$ source in bottom sediments due to its complete evaporation in the past. Based on the paucity of ClO$_3^-$ and ClO$_4^-$ in the deep waters, this lake appears to have supported ClO$_3^-$ and ClO$_4^-$ reduction at least back to the last draw down event. ClO$_3^-$ and ClO$_4^-$ concentrations in Lake Bonney are the highest of all the lakes reflecting the lake’s greater age and concentration of Cl$^-$. Similar to NO$_3^-$, ClO$_4^-$ appears to be stable in the East Lobe and its concentration is highly correlated to Cl$^-$ concentration. It is even possible that some ClO$_4^-$ at depth is a remnant of the initial seawater that formed Lake Bonney. In the West Lobe ClO$_3^-$ and ClO$_4^-$ appear stable at depths above the chemocline but have or are experiencing reduction at the deepest depth similar to NO$_3^-$. Finally the concentrations of ClO$_3^-$ and ClO$_4^-$ are well correlated except in cases where reduction has occurred.

These lakes provide an excellent case study for ClO$_3^-$ and ClO$_4^-$ biotransformation in pristine extreme environments. Given their low concentrations, high solubility, and lack of any in situ generation mechanisms, they may offer a sensitive means to study ongoing biological activity in the lakes, and the addition of ClO$_4^-$ stable isotope evaluation could provide further clues as to the geochemical history of the lake water. Finally, ClO$_3^-$ and ClO$_4^-$ biogeochemistry in Antarctic ice-covered lakes may represent an excellent analog for similar processes in ice-covered lakes on Mars in the past (McKay and Davis, 1991), or even in more recent times, especially given the discovery of relatively large amounts of ClO$_4^-$ in the Martian soil (Hecht et al., 2009).
2.5 Evaluate Cl and O Stable Isotope Signatures of UV and O3 Generated Perchlorate

2.5.1 Background

As previously described, natural production of ClO$_4^-$ has been linked to atmospheric processes involving the oxidation of Cl and oxy-chlorine (ClO$_x$) species by ultraviolet (UV) radiation and ozone (O$_3$) (Kang et al., 2006, 2008, 2009; Rao et al., 2010, Wang et al., 2011). The pathways for ClO$_4^-$ formation are ambiguous and not well-defined, this more so for production of ClO$_4^-$ by O$_3$ oxidation than by photo-oxidation, of which there have been more proposed pathways. Although the actual ClO$_4^-$ production pathways are still unknown, studies by our group and others (Rao et al., 2010, 2012; Kang et al., 2009) have identified some of the reactants responsible for ClO$_4^-$ formation by both UV and O$_3$ as well as some of the suspected intermediates and other major products. Formation of the ClO$_4^-$ is considered dependent upon many factors including: exposure time, mass of reactant, pH, type of system (dry vs. aqueous), surface material, and in the case of UV, the light source (wavelength) (Kang et al., 2006, 2008, 2009; Rao et al., 2010, Wang et al., 2011). Along with oxidation of oxy-chlorine species, production of ClO$_4^-$ has also been observed as a result of reactions involving chlorine monoxide (ClO) and chlorine dioxide (ClO$_2$) radicals on the surface of sulfuric acid (Jaegle et al., 1996). The factors affecting ClO$_4^-$ formation can all contribute to fractionation effects that can lead to the variation observed in natural ClO$_4^-$ isotopic compositions.

At present there are no studies related to the potential for different ClO$_4^-$ formation pathways to produce distinct variations in ClO$_4^-$ isotopic composition. The purpose of this study was to evaluate the potential for different formation processes to impact the stable isotopic composition of ClO$_4^-$ produced from UV or O$_3$ oxidation of Cl and oxy-chlorine species. Isotopic compositions for water (H$_2$O), O$_3$, ClO$_4^-$, starting materials, and major end products were determined. The isotopic data were compared to the known groups of natural ClO$_4^-$ isotopic compositions to determine if different ClO$_4^-$ formation processes contribute to the variation in natural ClO$_4^-$ isotopic compositions observed.

2.5.2 Methods

2.5.2.1 Preparation of Reactant Species

Aqueous solutions of hypochlorite (OCl$^-$), Cl$^-$, and chlorite (ClO$_2^-$) were prepared from calcium hypochlorite (Ca(OCl)$_2$; available chlorine ~65%, Sigma-Aldrich), sodium chloride (NaCl; ACS reagent, 99 + %; Sigma-Aldrich), and sodium chlorite (NaClO$_2$; technical grade, 80 %; Sigma-Aldrich) salts. All Ca(OCl)$_2$ solutions were filtered through a 0.2µm filter (PVDF Membrane, Millipore) before being used in the O$_3$ and photo-oxidation experiments. Concentrations for reactant species were based on ClO$_4^-$ yields from previous studies (Kang et al., 2008; Rao et al., 2010, 2012; Wang et al., 2011).
The aqueous chlorine dioxide (ClO₂) solutions were prepared according to a standard iodometric method (SMEWW, 1998) by use of a gas generating and absorption system, with minor modifications (Figure 2.5.1). The aspirator flask was filled with 300 mL distilled deionized (DDI) water, the gas-generating bottle with 750 mL DDI water and 10 g NaClO₂, the two scrubber bottles (used to capture impurities) with flaked NaClO₂ crystals, and the collecting bottle with 1500 mL DDI water. The collecting bottle was wrapped in aluminum foil to avoid the penetration of light in order to minimize the photodegradation of ClO₂ (aq). Before being connected to the gas-generating and absorption system the collecting bottle was chilled in the freezer and then placed in a bucket of ice so that better dissolution of ClO₂ occurred. The ClO₂ was generated by bubbling a smooth current of purified compressed air through the system and by adding 2 mL of H₂SO₄ in 5 mL increments through 5 minute intervals.
Figure 2.5.1 Chlorine dioxide gas-generating absorption system.
2.5.2.2 Ozone Experiments
Ozone experiments were conducted according to methods by Rao et al., 2010 with minor modifications. Ozone was produced by passing pure oxygen (O2-purity > 99.999%) through an adjustable corona discharge O3 generator (Model OL80W, Ozone Services) and was bubbled through aqueous solutions of ClO2, ClO2−, OCl−, and Cl− (Table 2.5.1 and Figure 2.5.2)

Table 2.5.1. Experimental Conditions for O₃ oxidation of various Cl/ClOₓ species.

<table>
<thead>
<tr>
<th>Replicate #</th>
<th>Reactant Species</th>
<th>Species Mass (mg)</th>
<th>Volume (L)</th>
<th>Exposure Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dry Cl⁻</td>
<td>514 041</td>
<td>N/A</td>
<td>~ 1 month</td>
</tr>
<tr>
<td>1</td>
<td>Cl⁻ (aq)</td>
<td>211 200</td>
<td>32</td>
<td>~ 9 months</td>
</tr>
<tr>
<td>2</td>
<td>Cl⁻ (aq)</td>
<td>747 000</td>
<td>18</td>
<td>~ 9 months</td>
</tr>
<tr>
<td>1</td>
<td>OCl⁻ (aq)</td>
<td>13 000</td>
<td>1.8</td>
<td>~ 1 day</td>
</tr>
<tr>
<td>2</td>
<td>OCl⁻ (aq)</td>
<td>8 640</td>
<td>0.5</td>
<td>~ 1 day</td>
</tr>
<tr>
<td>3</td>
<td>OCl⁻ (aq)</td>
<td>11 782*</td>
<td>0.5</td>
<td>~ 1 day</td>
</tr>
<tr>
<td>1</td>
<td>ClO₂⁻ (aq)</td>
<td>4 650</td>
<td>0.5</td>
<td>~ 2 – 20 hrs</td>
</tr>
<tr>
<td>2</td>
<td>ClO₂⁻ (aq)</td>
<td>4 133</td>
<td>0.5</td>
<td>~ 2 – 20 hrs</td>
</tr>
<tr>
<td>1</td>
<td>ClO₂ (aq)</td>
<td>38</td>
<td>0.5</td>
<td>~ 1.5 hrs</td>
</tr>
<tr>
<td>2</td>
<td>ClO₂ (aq)</td>
<td>71</td>
<td>1</td>
<td>~ 1.5 hrs</td>
</tr>
</tbody>
</table>

* Value is an approximation as solution was measured long after it was made
Generation of ClO$_4^-$ by O$_3$ oxidation of dry Cl$^-$ (NaCl; Certified ACS, ≥ 99%; Fisher Chemical) was also performed. Ozone was passed through approximately 50 glass tubes containing ± 10 grams NaCl each and connected in series by Teflon tubing (Figure 2.5.3). The flow rate of O$_3$ was maintained throughout the experiments by a gas flow controller (Model GFC-17; Aalborg) set closely at 5 mL/min. All experiments, minus the dry Cl$^-$, were performed in duplicate (Table 2.5.1). Starting and ending solutions were analyzed for Cl$^-$, OCl$^-$, ClO$_2^-$, ClO$_2$, ClO$_3^-$, and ClO$_4^-$. Figure 2.5.2. Experimental set-up for aqueous O$_3$ oxidation experiments.
2.5.2.3 UV Experiments

UV experiments were conducted according to methods by Rao et al., 2012b with minor modifications. Aqueous solutions of OCl⁻, ClO₂⁻, and ClO₂ were irradiated with UV light (λ = 350 nm) using a Rayonet photochemical chamber (Model RPR-200; Southern New England Ultraviolet Co., Brandford, CT) equipped with 8 UV lamps (Table 2.5.2 and Figure 2.5.4). Erlenmeyer flasks containing the solutions and stoppered with silicone caps were placed inside the photochemical chamber until complete conversion of the reactant species (OCl⁻, ClO₂⁻, and ClO₂) was observed. All experiments were performed in duplicate and starting and ending solutions were analyzed for Cl⁻, OCl⁻, ClO₂⁻, ClO₂, ClO₃⁻, and ClO₄⁻.

Figure 2.5.3. Experimental set-up for O₃ oxidation of dry Cl⁻.
Table 2.5.2. Experimental conditions for UV oxidation of various ClO$_x$ species.

<table>
<thead>
<tr>
<th>Replicate #</th>
<th>Reactant Species</th>
<th>Species Mass (mg)</th>
<th>Volume (L)</th>
<th>Wavelength $\lambda$</th>
<th>Exposure Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OCl$^-$ (aq)</td>
<td>20 000</td>
<td>0.8</td>
<td>350 nm</td>
<td>~ 5 – 10 hrs</td>
</tr>
<tr>
<td>2</td>
<td>OCl$^-$ (aq)</td>
<td>36 000</td>
<td>1</td>
<td>350 nm</td>
<td>~ 5 – 10 hrs</td>
</tr>
<tr>
<td>1</td>
<td>ClO$_2^-$ (aq)</td>
<td>8 000</td>
<td>0.3</td>
<td>350 nm</td>
<td>~ 1 – 3 hrs</td>
</tr>
<tr>
<td>2</td>
<td>ClO$_2^-$ (aq)</td>
<td>10 000</td>
<td>1</td>
<td>350 nm</td>
<td>~ 1 – 3 hrs</td>
</tr>
<tr>
<td>1</td>
<td>ClO$_2$ (aq)</td>
<td>2 664</td>
<td>37</td>
<td>350 nm</td>
<td>~ 30 min – 3 hrs</td>
</tr>
<tr>
<td>2</td>
<td>ClO$_2$ (aq)</td>
<td>1 941</td>
<td>23.7</td>
<td>350 nm</td>
<td>~ 30 min – 3 hrs</td>
</tr>
</tbody>
</table>

* Value is an approximation as solution was measured long after it was made

Figure 2.5.4. Experimental set-up for UV oxidation experiments using the Rayonet photochemical chamber.
2.5.2.4 Sample Analysis

Perchlorate and ClO₃⁻ concentrations in starting and ending solutions were determined using sequential ion chromatography-mass spectrometry/mass spectrometry (IC-MS/MS) as described in Rao et al. (2007). Concentrations of Cl⁻ and ClO₂⁻ were determined using ion-chromatography (IC) (Dionex LC20 enclosure) following U.S. EPA Methods 300.0 (USEPA, 1993) and 326.0 (Thomas et al., 2013) with minor modifications. Chloride was analyzed using a Dionex IonPac AS14A column (4 x 250 mm) and an 8 mM sodium carbonate (Na₂CO₃)/1 mM sodium bicarbonate (NaHCO₃) eluent while ClO₂⁻ was analyzed using a Dionex AS9-HC column and a 9 mM Na₂CO₃ eluent. The limit of detection (LOD) for Cl⁻ and ClO₂⁻ was 0.5 mg/L.

Hypochlorite was measured as total Cl₂ (total Cl₂ = Cl₂ + HOCl + OCl⁻) by buffering solution samples at pH 8.5 and spiking them with excess iodide (I⁻) to produce a tri-iodide species (I₃⁻) that could be measured using a spectrophotometer (DU® Series 700, Beckman Coulter®) at 351 nm (ε = 2.54 ± 0.02) x 10⁴ M⁻¹cm⁻¹). The reaction stoichiometry between Cl₂ and I₃⁻ was used to determine OCl⁻ concentration. The ClO₂⁻ in solutions was determined by both UV spectrometry at 359 nm (ε = 1230 ± 10 M⁻¹cm⁻¹) and iodometric titration with 0.025 N sodium thiosulfate (Na₂S₂O₃) (SMEWW, 1998).

2.5.2.5 Perchlorate Extraction and Purification

All ending solutions were passed through a Purolite® A530E resin column at a constant flow rate until at least 2 mg of ClO₄⁻ was collected (Gu et al., 2011). Ending solutions containing high amounts of ClO₃⁻ were first reduced so that their presence did not interfere with the uptake of ClO₄⁻ by the resin column. Reduction was achieved by first purging the solutions with helium (He) to remove any remaining oxidants and then by adding elemental nickel (Ni) and purging the solutions with hydrogen gas (H₂) to reduce the high level species. Resin columns were preserved with hydrochloric acid (HCl) at a pH of 2 and were sent to Oak Ridge National Laboratory for elution and purification, as described in Gu et al. (2011) and Hatzinger et al. (2011).

The elution process consisted of removing the Purolite® A530E resin containing the absorbed ClO₄⁻ from the column, rinsing it with ultrapure DDI water in an ultrasonic bath, transferring it into a preparative glass chromatography column (100 mL volume), and then washing it approximately with 5 bed volumes (BV) of 4 M HCl to remove any absorbed impurities (i.e. NO₃⁻, organics, carbonates) (Hatzinger et al., 2011). The adsorbed ClO₄⁻ was eluted from the preparative glass column with a combination of 1 M FeCl₃ and 4 M HCl. The FeCl₃-HCl eluent solution (containing the eluted ClO₄⁻) contains high concentrations of organics, Fe, and other anions aside from ClO₄⁻, the eluent solution was neutralized with NaOH (pH ~ 9 – 10) to precipitate out the Fe and other impurities (Hatzinger et al., 2011). These precipitates were washed and centrifuged to collect a clear supernatant solution that was evaporated using a
vacuum concentration system to form precipitates (removed through filtration) until the ClO$_4^-$ concentration in solution was in excess of 3 mg/mL. The ClO$_4^-$ from the remaining purified solution was crystallized as cesium perchlorate (CsClO$_4$) by adding CsCl. A detailed version of the elution and purification method is described in Hatzinger et al. (2011).

2.5.2.6 Isotope Ratio Analyses

Oxygen isotope analyses were performed at the Reston Stable Isotope Laboratory of the U.S. Geological Survey in Reston, VA by high-temperature conversion of the purified ClO$_4^-$ salt (CsClO$_4$) to CO that was measured in continuous-flow mode by isotope-ratio mass spectrometry (CO-CFIRMS) and on O$_2$ produced by in vacuo decomposition of the ClO$_4^-$ salt (CsClO$_4$) analyzed in dual-inlet mode by isotope-ratio mass spectrometry (O2-DIIRMS) (Sturchio et al., 2007, 2011; Böhlke et al., 2005; Bao and Gu, 2004; Hatzinger et al., 2011). Oxygen isotope ratios are reported in units of δ$^{18}$O, δ$^{17}$O, and Δ$^{17}$O in terms of per mil (‰), defined as the deviation from the Vienna Standard Mean Ocean Water (δ$^{18}$OVSMOW = 0.0‰) (Equations 1-3).

$$
\delta^{18}O = \left[ \frac{n^{(18)O}}{n^{(16)O}} \right]_{sample} - 1 \times 1000
$$

(1)

$$
\delta^{17}O = \left[ \frac{n^{(17)O}}{n^{(16)O}} \right]_{sample} - 1 \times 1000
$$

(2)

$$
\Delta^{17}O = k = \left( \frac{1 + \delta^{17}O}{1 + \delta^{18}O} \right)^{\lambda} - 1 \times 1000
$$

(3)

Chlorine isotope ratios were analyzed at the Environmental Isotope Geochemistry Laboratory of the University of Illinois at Chicago by conversion of the CsClO$_4$ salt into methyl chloride (CH$_3$Cl) gas that was measured by ion-ratio mass spectrometry (IRMS) (Sturchio et al., 2007, 2011; Böhlke et al., 2005; Hatzinger et al., 2011). Perchlorate Cl isotope ratios are all reported in units of δ$^{37}$Cl in terms of per mil (‰), defined as the deviation from that of Standard Mean Ocean Chloride (SMOC) (Equation 4).
\[
\delta^{37} Cl = \left( \frac{\left[ n^{37}Cl \right]_{sample}}{\left[ n^{35}Cl \right]_{SMOC}} - 1 \right) \times 1000
\]  

(4)

2.5.3 Results

2.5.3.1 Mass Balance and Product Yields

A mass balance on initial and final amounts of Cl of ClO\textsubscript{x} species for UV and O\textsubscript{3} mediated ClO\textsubscript{4}\textsuperscript{-} generation experiments resulted in a Cl recovery of 81-116%, with one exception (Table 2.5.3). For one of three replicate experiments in which OCl\textsuperscript{-} was oxidized by O\textsubscript{3}, the total Cl recovery was only 31%. It is unclear whether the missing or excess Cl is related to analytical error due to the high concentrations of some species compared to others, or in the case of missing Cl perhaps due to production of Cl containing gasses that escaped the system (e.g. ClO\textsubscript{2}).

Production yields for ClO\textsubscript{x} species detected in final solutions were also evaluated (Table 2.5.3). ClO\textsubscript{4}\textsuperscript{-} production yields for the majority of experiments were within an order of magnitude of each other (0.01 – 0.15 %). The exceptions were the O\textsubscript{3} aqueous and dry Cl\textsuperscript{-} experiments, which had the lowest ClO\textsubscript{4}\textsuperscript{-} yields (note that not all Cl\textsuperscript{-} was reacted), and the O\textsubscript{3}-ClO\textsubscript{2} (aq) and one UV-ClO\textsubscript{2}\textsuperscript{-} experiment(s), which had the highest ClO\textsubscript{4}\textsuperscript{-} yields. It is unclear as to why one of the UV-ClO\textsubscript{2}\textsuperscript{-} replicates had a ClO\textsubscript{4}\textsuperscript{-} yield 10-fold higher than the other, but it could be related to this treatment’s higher initial ClO\textsubscript{2}\textsuperscript{-}concentration as suggested by Kang et al. (2006) and Rao et al. (2012b). This UV-ClO\textsubscript{2}\textsuperscript{-} replicate also had a different product distribution than its replicate, suggesting that the difference in ClO\textsubscript{4}\textsuperscript{-} yields may be due to different production mechanisms.

Oxidation of aqueous solutions of all starting ClO\textsubscript{x} species produced Cl\textsuperscript{-} and ClO\textsubscript{3}\textsuperscript{-} as major products with substantially smaller ClO\textsubscript{4}\textsuperscript{-} yields. Cl\textsuperscript{-} was the dominant product for UV and O\textsubscript{3} mediated oxidation of OCl\textsuperscript{-} and UV mediated oxidation of ClO\textsubscript{2}\textsuperscript{-}. ClO\textsubscript{3}\textsuperscript{-} was the major product for O\textsubscript{3} mediated oxidation of ClO\textsubscript{2}\textsuperscript{-} and UV and O\textsubscript{3} mediated oxidation of ClO\textsubscript{2} (aq). Except for the case of oxidation of solutions of ClO\textsubscript{2}\textsuperscript{-} or ClO\textsubscript{2}(aq) by O\textsubscript{3} for which the ClO\textsubscript{3}\textsuperscript{-} yield was > 80 %, yields of Cl\textsuperscript{-} and ClO\textsubscript{3}\textsuperscript{-} generally were within a factor of 2–3 for O\textsubscript{3} and UV mediated oxidation of aqueous solutions (Table 2.5.3). However, final products from the oxidation of Cl\textsuperscript{-} as a dry solid or in aqueous solution could not be evaluated as the majority of the Cl\textsuperscript{-} was unreacted at the end of the oxidation period. As such, a better representation of the product distribution for the O\textsubscript{3}-Cl\textsuperscript{-} (aqueous and dry) experiments would be the final molar ClO\textsubscript{4}\textsuperscript{-}/ClO\textsubscript{3}\textsuperscript{-} ratio, which for our study was estimated to be 0.0002 – 0.0003 and 5 for the aqueous and dry experiments, respectively. The O\textsubscript{3} oxidation of dry Cl\textsuperscript{-} was the only experimental condition for which ClO\textsubscript{4}\textsuperscript{-} was produced in excess of ClO\textsubscript{3}\textsuperscript{-}. Although the experimental conditions for our UV
and O₃ experiments were not the same as in previous studies, most of our yields were comparable to those reported in the same studies (Kang et al., 2006, 2008; Rao et al., 2010, 2012b; Wang et al., 2011).
Table 2.5.3. Cl amounts in ClOx species of initial and final solutions, Cl mass balance, and product yields for UV and O3 experiments.

### Ozone Experiments

<table>
<thead>
<tr>
<th>Reactant Species</th>
<th>Initial Solution</th>
<th>Final Solution</th>
<th>Mass Balance</th>
<th>Product Yields*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT mmol</td>
<td>OCl- mmol</td>
<td>ClO2- mmol</td>
<td>ClO2(aq) mmol</td>
</tr>
<tr>
<td>1-OCl-</td>
<td>140.8</td>
<td>252.7</td>
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### UV Experiments

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<th>Product Yields*</th>
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<td>OCl- mmol</td>
<td>ClO2- mmol</td>
<td>ClO2(aq) mmol</td>
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NM = Not measured
N/Y = No Yield

* Product Yields were calculated using the following equation: *Product Yield = (Final ClOx-Initial ClOx)/Main Reactant ClOx*
2.5.3.2 Ozone Experiment Isotopes

**Dry Chloride**
The $\delta^{37}\text{Cl}$ values of the initial dry Cl$^-$ salt and the final solution total Cl were indistinguishable (-0.1 ‰) (Figure 2.5.5). This is because the reactants and products were essentially composed of all Cl$^-$ and only a small amount of initial Cl$^-$ reacted. ClO$_4^-$ had $\delta^{37}\text{Cl}$ values ~ 3.5 ‰ lower than those of initial and final total Cl and Cl$^-$. 

$\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values of ClO$_4^-$ in the final solution were + 21 ‰ and +33 ‰, respectively. $\delta^{18}\text{O}$ values of final total Cl compounds (ClO$_3^-$ and ClO$_4^-$) could not be measured because of high total Cl/O ratios.

**Aqueous Chloride**
Initial total Cl ($\approx$ Cl$^-$), final total Cl (Cl$^- + $ ClO$_3^- + $ ClO$_4^-$), and final Cl$^-$ had similar $\delta^{37}\text{Cl}$ values in both aqueous Cl$^-$ experiments (Figure 2.5.6). ClO$_4^-$ had $\delta^{37}\text{Cl}$ values ~ 7 ‰ higher than those of initial and final total Cl and Cl$^-$. 

The $\delta^{18}\text{O}$ values of final total O ($\approx$ ClO$_3^-$) were ~ 60 – 75 ‰ lower than those of ClO$_4^-$. The $\Delta^{17}\text{O}$ values of final total O ($\approx$ ClO$_3^-$) were greater than zero but much lower (~ 25 ‰ to 28 ‰) than ClO$_4^- \Delta^{17}\text{O}$ values.
Figure 2.5.5. Stable isotopic composition of initial and final species for O₃ oxidized dry Cl⁻. Replicate experiments are represented by the different colors and different symbols represent the Cl and O of ClOₓ species present in initial and final solutions, where Ti = total initial, Tf = total final, I = initial, and F = final. Note that the horizontal axis indicates the amount of Cl (for δ³⁷Cl) and the amount of O (for δ¹⁸O and Δ¹⁷O) in the initial and final experimental solutions.
Figure 2.5.6. Stable isotopic composition of initial and final species for O₃ oxidized solutions of Cl⁻ (aq). Replicate experiments are represented by the different colors and different symbols represent the Cl and O of ClOₓ species present in initial and final solutions, where Tᵢ = total initial, Tₐ = total final, I=initial, and F=final. Note that the horizontal axis indicates the amount of Cl (for δ³⁷Cl) and the amount of O (for δ¹⁸O and Δ¹⁷O) in the initial and final experimental solutions.
**Hypochlorite**

Not all initial and final $\delta^{37}\mathrm{Cl}$, $\delta^{18}\mathrm{O}$, and $\Delta^{17}\mathrm{O}$ values were measured for each O$_3$ oxidized replicate OCl$^-$ experiment (Figure 2.5.7). The $\delta^{37}\mathrm{Cl}$ value (+ 2.56 ‰) of the initial total Cl (Cl$^-$ + ClO$_3^-$ + ClO$_4^-$) was similar (< 2.5 ‰) to the final total Cl (OCl$^-$ + Cl$^-$), initial Cl$^-$, and final Cl$^-$ $\delta^{37}\mathrm{Cl}$ values. Compared to initial total Cl (OCl$^-$ + Cl$^-$), the estimated $\delta^{37}\mathrm{Cl}$ value (using a mass balance) for produced ClO$_3^-$ and the $\delta^{37}\mathrm{Cl}$ values of ClO$_4^-$ of all the experiments were on average ~ 15 ‰ higher.

Final total O $\delta^{18}\mathrm{O}$ values were similar between all replicates as were final ClO$_4^-$ $\delta^{18}\mathrm{O}$ values, which were (32 ‰ – 47 ‰) higher than the final total O (essentially all ClO$_3^-$). Initial total O $\delta^{18}\mathrm{O}$ values of two of the replicate experiments were similar and only slightly lower than final ClO$_4^-$ $\delta^{18}\mathrm{O}$ values. However, the third replicate experiment had a much lower initial total O $\delta^{18}\mathrm{O}$ value (~ 24 ‰), which is odd given the similar $\delta^{18}\mathrm{O}$ values for the final total O for all treatments and for final ClO$_4^-$ for all treatments. This may suggest that the very low initial total $\delta^{18}\mathrm{O}$ values for the third replicate may be invalid. $\Delta^{17}\mathrm{O}$ values were similar for initial total O (OCl$^-$ + ClO$_3^-$) and final total O (≈ ClO$_3^-$), with less than 1.5 ‰ variation. On the contrary, ClO$_4^-$ $\Delta^{17}\mathrm{O}$ values were much higher (15 ‰ – 20 ‰) compared to final ClO$_3^-$ and initial and final total O.
Figure 2.5.7. Stable isotopic composition of initial and final species for O$_3$ oxidized solutions of OCl$^-$ (aq). Replicate experiments are represented by the different colors and different symbols represent the Cl and O of ClO$_x$ species present in initial and final solutions, where T$_i$ = total initial, T$_f$ = total final, I = initial, and F = final. Note that the horizontal axis indicates the amount of Cl (for $\delta^{37}$Cl) and the amount of O (for $\delta^{18}$O and $\Delta^{17}$O) in the initial and final experimental solutions.
**Chlorite**
The δ\(^{37}\)Cl values of initial total Cl (ClO\(_2^- + \) Cl\(^-\)) and final total Cl (Cl\(^- +\) ClO\(_3^-\)) were similar (< 0.5 ‰) for all ClO\(_2^-\) replicates (Figure 2.5.8). Likewise, δ\(^{37}\)Cl values of initial and final Cl\(^-\) were similar to one another (< 2 ‰ difference), but were lower than the δ\(^{37}\)Cl values of the initial total Cl (ClO\(_2^- + \) Cl\(^-\)) by 4 ‰ - 6 ‰. Estimated (by mass balance) δ\(^{37}\)Cl values for initial ClO\(_2^-\) and final ClO\(_3^-\) were similar to each other and higher than initial total Cl δ\(^{37}\)Cl values by 1.5‰ - 2 ‰. δ\(^{37}\)Cl values of ClO\(_4^-\) (+ 5 ‰ - + 6 ‰) were higher than all other species.

Total O in the initial solution (≈ ClO\(_2^-\)) and final solution (≈ ClO\(_3^-\)) had similar δ\(^{18}\)O values, but these δ\(^{18}\)O values were lower (~ 3 ‰ - 6 ‰) than those of produced ClO\(_4^-\). In the initial solution the Δ\(^{17}\)O values of total O (≈ ClO\(_2^-\)) were lower than the Δ\(^{17}\)O values of the total O (≈ ClO\(_3^-\)) and ClO\(_4^-\) in final solution by ~ 3 ‰ and 12 ‰, respectively.

**Chlorine Dioxide**
ClO\(_2\) (aq) initial and final solution isotopes are not available, except for final ClO\(_4^-\) (Figure 2.5.9). The δ\(^{37}\)Cl value of ClO\(_4^-\) (~ +16 ‰) was only determined for one experiment. The δ\(^{18}\)O value of ClO\(_4^-\) varied between the experiments, with one of the replicates being 4 ‰ higher in δ\(^{18}\)O. Δ\(^{17}\)O values of ClO\(_4^-\) were similar between both experiments at approximately + 16.5 ‰.
Figure 2.5.8. Stable isotopic composition of initial and final species for O₃ oxidized solutions of ClO₂⁻ (aq). Replicate experiments are represented by the different colors and different symbols represent the Cl and O of ClOₓ species present in initial and final solutions, where T₁ = total initial, T_F = total final, I = initial, and F = final. Note that the horizontal axis indicates the amount of Cl (for δ³⁷Cl) and the amount of O (for δ¹⁸O and Δ¹⁷O) in the initial and final experimental solutions.
Figure 2.5.9. Stable isotopic composition of initial and final species for O₃ oxidized solutions of ClO₂ (aq). Replicate experiments are represented by the different colors and different symbols represent the Cl and O of ClOₓ species present in initial and final solutions, where Tᵢ = total initial, Tᵢ = total final, I = initial, and F = final. Note that the horizontal axis indicates the amount of Cl (for δ³⁷Cl) and the amount of O (for δ¹⁸O and Δ¹⁷O) in the initial and final experimental solutions.
2.5.3.3 UV Experiment Isotopes

Hypochlorite
Regardless of the starting OCl− mass and production yields, the isotopic compositions (δ\textsuperscript{37}Cl, δ\textsuperscript{18}O, and Δ\textsuperscript{17}O) of the reactants and products from photolysis (λ=350 nm) of OCl− solutions were essentially the same between both experiments (Figure 2.5.10). The δ\textsuperscript{37}Cl of initial total Cl (OCl− + Cl− + ClO\textsubscript{3}−), final total Cl (Cl− + ClO\textsubscript{3}− + ClO\textsubscript{4}−), initial Cl−, and final Cl− did not vary (<1 ‰). δ\textsuperscript{37}Cl in ClO\textsubscript{4}− and ClO\textsubscript{3}− (based on mass balance) were higher (~15 ‰ and 2 ‰, respectively) than initial and final total Cl or Cl−.

The total O of ClO\textsubscript{x} species in the final solution, which was almost solely composed of ClO\textsubscript{3}−, had δ\textsuperscript{18}O values approximately 11 ‰ − 15 ‰ lower than those of total O for the initial total ClO\textsubscript{x} (~ OCl− + Cl−). Contrarily, ClO\textsubscript{4}− δ\textsuperscript{18}O was 7 ‰ higher than δ\textsuperscript{18}O of initial total ClO\textsubscript{x} (~ OCl− + Cl−). Δ\textsuperscript{17}O values were essentially the same (-0.33 ‰ to +0.45 ‰) among all major species, both initial and final (ClO\textsubscript{4}−, ClO\textsubscript{3}−, and total O in ClO\textsubscript{x}).
Figure 2.5.10. Stable isotopic composition of initial and final species for solutions of OCl\textsuperscript{−} (aq) exposed to 350 nm irradiation. Replicate experiments are represented by the different colors and different symbols represent the Cl and O of ClO\textsubscript{x} species present in initial and final solutions, where T\textsubscript{T} = total initial, T\textsubscript{F}= total final, I=initial, and F=final. Note that the horizontal axis indicates the amount of Cl (for δ\textsuperscript{37}Cl) and the amount of O (for δ\textsuperscript{18}O and Δ\textsuperscript{17}O) in the initial and final experimental solutions.
**Chlorite**

There was little variation (< ± 2 ‰) in the δ^{37}Cl values of initial total Cl (ClO_3^- + ClO_2^- + Cl^-), final total Cl (Cl^- + ClO_3^- + ClO_4^-), and final Cl^- for photolysis (λ = 350 nm) of ClO_2^- solutions (Figure 2.5.11). δ^{37}Cl values of ClO_4^- and estimated ClO_3^- (based on mass balance) were higher (~ 24 ‰ and 4 ‰, respectively) compared to final Cl^− and total Cl in initial and final solution.

There were small changes (< 3 ‰) in δ^{18}O values of initial total O (~ ClO_2^-) and final total O, which was primarily composed of ClO_3^- . Compared to initial total O (~ ClO_2^-), the δ^{18}O value for ClO_4^- was higher (~ 42 ‰) and estimated δ^{18}O value for ClO_3^- was lower (~ 3 ‰). No difference in Δ^{17}O values between initial (~ ClO_2^-) and final total O (~ ClO_3^-) was observed, but the Δ^{17}O value of ClO_4^- was smaller (< 2 ‰).

**Chlorine Dioxide**

Isotopic compositions of initial ClO_2 (aq) and final ClO_3^- (aq) were not able to be measured. The δ^{37}Cl values (~ + 18 ‰ and + 20 ‰), δ^{18}O values (~ + 9 ‰ and + 13 ‰), and Δ^{17}O values of ClO_4^- (~ 0 ‰) were similar between replicates (Figure 2.5.12).
Figure 2.5.11. Stable isotopic composition of initial and final species for solutions of ClO$_2$ (aq) exposed to 350 nm irradiation. Replicate experiments are represented by the different colors and different symbols represent the Cl and O of ClO$_x$ species present in initial and final solutions, where T$_i$ = total initial, T$_F$ = total final, I = initial, and F = final. Note that the horizontal axis indicates the amount of Cl (for $\delta^{37}$Cl) and the amount of O (for $\delta^{18}$O and $\Delta^{17}$O) in the initial and final experimental solutions.
Figure 2.5.12. Stable isotopic composition of initial and final species for solutions of ClO₂ (aq) exposed to 350 nm irradiation. Replicate experiments are represented by the different colors and different symbols represent the Cl and O of ClOx species present in initial and final solutions, where T₁ = total initial, T₂ = total final, I = initial, and F = final. Note that the horizontal axis indicates the amount of Cl (for δ³⁷Cl) and the amount of O (for δ¹⁸O and Δ¹⁷O) in the initial and final experimental solutions.
2.5.4 Discussion

2.5.4.1 Overall Observations

Perchlorate formed from UV and O₃ mediated oxidation processes generally had higher δ³⁷Cl and δ¹⁸O values than all other ClOₓ species (initial and final), with a few exceptions. In some cases, there was not enough O in the products for an IR-MS analysis to be made and in others, initial ClOₓ species were simply not measured. In only one of the experiments (dry Cl⁻), for which ClO₄⁻ isotopes were measured, was the δ³⁷Cl of the initial Cl⁻ higher than in ClO₄⁻.

The same trend was observed for δ¹⁸O values of produced ClO₄⁻ compared to those of the H₂O used in the experiments. With the exception of the UV OCl⁻ experiments, H₂O typically bore a lower δ¹⁸O value than O in ClO₄⁻. The opposite was observed for the δ¹⁸O of O₃ generated in the lab versus δ¹⁸O of ClO₄⁻ from O₃ experiments. The δ¹⁸O of O₃ (+ 124 ‰ ± 6 ‰) produced in the lab via the O₃ generator was always substantially higher than δ¹⁸O of produced ClO₄⁻, for which the highest δ¹⁸O value reported was ~ + 55 ‰.

The Δ¹⁷O of ClO₄⁻ varied between the oxidation methods. In most cases, the Δ¹⁷O values of ClO₄⁻ were below the Δ¹⁷O value of lab generated O₃ (+ 24.5 ‰). The exceptions were the dry and aqueous Cl⁻ experiments for which values ranged from + 30 ‰ to + 33 ‰.

2.5.4.2 O₃ vs UV ClO₄⁻ Isotopes

There was a discernible difference between the Δ¹⁷O of ClO₄⁻ produced by O₃ mediated processes and the Δ¹⁷O of ClO₄⁻ produced by UV mediated processes (Figures 2.5.13 and 2.5.14). While the Δ¹⁷O of UV ClO₄⁻ was typically around zero (- 1.55 ‰ to + 1.33 ‰), the Δ¹⁷O of O₃ ClO₄⁻ (+ 12 ‰ to + 33 ‰) was substantially higher, indicating the source of O for oxidation of Cl in ClOₓ precursor and intermediate species was different between UV and O₃ experiments.

Like Δ¹⁷O of ClO₄⁻, ClO₄⁻ formed through O₃ mediated oxidation reactions tended to have higher δ¹⁸O values than ClO₄⁻ formed though oxidation reactions produced by photolysis. The exception being the UV ClO₂⁻ experiment, which had a ClO₄⁻ with a higher δ¹⁸O value than the ClO₄⁻ produced from O₃ oxidation of the same precursor species. In the case of δ³⁷Cl, the δ³⁷Cl of UV produced ClO₄⁻ was always higher than the δ³⁷Cl of ClO₄⁻ produced from O₃ oxidation of the same precursor species.
Figure 2.5.13. Stable isotopic compositions ($\delta^{37}\text{Cl}$, $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$) of reactant species and final ClO$_4^-$ for O$_3$ oxidized solutions. The vertical dashed light blue and pink lines represent the $\delta^{18}\text{O}$ value for initial and final H$_2$O, respectively. The thick dark maroon dashed line represents the average $\delta^{18}\text{O}$ value for O$_3$ and the thinner dashed lines represent the standard deviation from the average $\delta^{18}\text{O}$ value. Replicate experiments are represented by the different colors and different symbols represent the reactant species and ClO$_4^-$. 
Figure 2.5.14. Stable isotopic composition (δ\(^{37}\)Cl, δ\(^{18}\)O, Δ\(^{17}\)O) of reactant species and final ClO\(_4^−\) for UV oxidized solutions. Replicate experiments are represented by the different colors and different symbols represent the reactant species and ClO\(_4^−\).
2.5.4.3 $\Delta^{17}$O vs Precursor ClO$_x$ Species

There appears to be an inverse relationship between $\Delta^{17}$O values of ClO$_4^-$ formed by O$_3$ mediated oxidation processes and the oxidation state of the Cl in the precursor ClO$_x$ species (Figure 2.5.15). With the exception of the O$_3$ ClO$_2^-$ experiments, $\Delta^{17}$O values of ClO$_4^-$ in O$_3$ experiments decreased with increasing oxidation state of ClO$_x$ species (Figure 2.5.15f). This trend was not as obvious for the UV experiments (Figure 2.5.15c) as no experiments were conducted with Cl$^-$ (aqueous or dry), but there was a consistency with behavior of $\Delta^{17}$O values in ClO$_4^-$ with the rest of the precursor species (OCl$^-$, ClO$_2^-$, and ClO$_2$) between both oxidation methods. In both UV and O$_3$ (not considering Cl$^-$), ClO$_4^-$ formed from oxidation of ClO$_2^-$ had the highest $\Delta^{17}$O values, followed by ClO$_4^-$ formed from OCl$^-$, and lastly ClO$_4^-$ formed from ClO$_2^-$. The $\Delta^{17}$O values of ClO$_4^-$ produced from UV experiments, however, were so close to zero that we cannot be sure that what we observed is actually a trend, or rather an error associated with the measurement method.

2.5.4.4 $\delta^{18}$O vs Precursor ClO$_x$ Species

The relationship between $\delta^{18}$O values of ClO$_4^-$ formed by O$_3$ mediated oxidation processes and the oxidation state of the Cl in the precursor ClO$_x$ species parallels that of the $\Delta^{17}$O values of ClO$_4^-$ and oxidation state of the Cl in the precursor ClO$_x$ species in O$_3$ experiments (Figure 2.5.15e). This seems logical as the mass-independent $\Delta^{17}$O anomaly is calculated based on measured $\delta^{18}$O and $\delta^{17}$O values.

No connection was observed between oxidation state of the Cl in the precursor ClO$_x$ species and $\delta^{18}$O values of ClO$_4^-$ in the UV experiments. Nevertheless, it should be noted that ClO$_4^-$ formed from UV oxidation of ClO$_2^-$ had exceedingly high $\delta^{18}$O values, almost as high as those reported for ClO$_4^-$ formed from O$_3$ oxidation of Cl$^-$ (aq) (Figure 2.5.15b). On the contrary, $\Delta^{17}$O values of ClO$_4^-$ produced by UV oxidation of ClO$_2^-$ were lower than any of the other UV experiments (Figure 2.5.12c).
Figure 2.5.15. Relationship between $\delta^{37}$Cl of final ClO$_4^-$ produced by UV and O$_3$ mediated oxidation of Cl$^-$ (s), Cl$^-$ (aq), OCl$^-$ (aq), ClO$_2^-$ (aq), ClO$_2^-$ (aq) and the oxidation state of Cl in precursor ClO$_x$ species. UV experiment data (red bars) are presented in graphs a, b, and c while the O$_3$ experiment data (blue bars) are presented in graphs d, e, and f. The name of the ClO$_x$ species associated each bar is written on or on top of each bar. Data with no replicates do not include error bars.
2.5.4.5 \( \delta^{37}\text{Cl} \) vs Precursor ClO\(_x\) Species

No interdependence was observed between \( \delta^{37}\text{Cl} \) values of ClO\(_4^-\) formed from O\(_3\) and UV mediated oxidation reactions with ClO\(_x\) species and the oxidation state of the Cl in the precursor ClO\(_x\) species. In the UV experiments, ClO\(_4^-\) formed from UV oxidation of ClO\(_2^-\) was enriched in \( \delta^{37}\text{Cl} \) compared to ClO\(_4^-\) from UV oxidation of OCl\(^-\) and ClO\(_2\)(aq) (Figure 2.5.15a). In contrast, the \( \delta^{37}\text{Cl} \) of ClO\(_4^-\) formed from O\(_3\) oxidation of ClO\(_2^-\) was depleted in \( \delta^{37}\text{Cl} \) compared to ClO\(_4^-\) from UV oxidation Cl\(^-\)(aq), OCl\(^-\), and ClO\(_2\)(aq), but not dry Cl\(^-\) (Figure 2.5.15d). Although the Cl in both aqueous and dry Cl\(^-\) have the same oxidation number, O\(_3\) oxidation of dry Cl\(^-\) resulted in ClO\(_4^-\) with negative \( \delta^{37}\text{Cl} \) values, much lower than those of ClO\(_4^-\) formed from aqueous Cl\(^-\) or other species (OCl\(^-\), ClO\(_2^-\), ClO\(_2\)(aq)) (Figure 2.5.15d).

2.5.4.6 Dry vs Aqueous Chloride

There was a clear difference between ClO\(_4^-\) formed from O\(_3\) oxidation of dry Cl\(^-\) and Cl\(^-\) in aqueous solution (Figure 2.5.15). Perchlorate formed from O\(_3\) oxidation of dry Cl\(^-\) was depleted in \( \delta^{37}\text{Cl} \) and \( \delta^{18}\text{O} \) compared to ClO\(_4^-\) formed from O\(_3\) oxidation of Cl\(^-\)(aq), but was enriched in \( \Delta^{17}\text{O} \). This is inconsistent with expected \( \Delta^{17}\text{O} \) values of ClO\(_4^-\) from O\(_3\) oxidation of dry Cl\(^-\) given the measured \( \delta^{18}\text{O} \) values of the ClO\(_4^-\). O\(_3\) mediated oxidation reactions with dry Cl\(^-\) also yielded ClO\(_4^-\) containing the only negative \( \delta^{37}\text{Cl} \) values measured in all of our experiments (including both O\(_3\) and UV oxidation of ClO\(_x\) species). Based on these results, oxidation reactions occurring on solid surfaces do not fractionate species in the same way as they would if the species were dissolved in aqueous media.

2.5.4.7 ClO\(_3^-\) Isotopes

Estimated \( \Delta^{17}\text{O} \) values of chlorate (based on mass balance) formed from both UV and O\(_3\) processes were always lower than \( \Delta^{17}\text{O} \) values of ClO\(_4^-\). \( \Delta^{17}\text{O} \) values of ClO\(_3^-\) from UV experiments were consistently below zero, whereas in the O\(_3\) experiments \( \Delta^{17}\text{O} \) of ClO\(_3^-\) were consistently above zero, but not by much (+ 0.8 ‰ to + 4 ‰). The small \( \Delta^{17}\text{O} \) anomaly observed in the ClO\(_3^-\) produced by O\(_3\) oxidation of ClO\(_x\) species suggests that although a minor contribution of O\(_3\)-O exists in the formation of ClO\(_3^-\), that O atoms in ClO\(_3^-\) are mostly not from O\(_3\). The limited contribution of O\(_3\)-O in the ClO\(_3^-\) structure may be indicative of reaction mechanisms for which the early reaction steps are similar for both ClO\(_4^-\) and ClO\(_3^-\) formation, but then diverge later in the process to form ClO\(_4^-\) and ClO\(_3^-\) separately.

Calculated final product ClO\(_3^-\)/ClO\(_4^-\) ratios in the O\(_3\) experiments ranged from 0.18 – 4431, with the higher and lower end of this range corresponding to the O\(_3\) oxidation of dry Cl\(^-\) and Cl\(^-\)(aq), respectively. Of the O\(_3\) experiments for which ClO\(_3^-\) \( \Delta^{17}\text{O} \) values were estimated (Cl\(^-\)(aq), OCl\(^-\), and ClO\(_2^-\)), no clear trend was discernible between ClO\(_3^-\)/ClO\(_4^-\) final product ratios and the \( \Delta^{17}\text{O} \) values of ClO\(_3^-\). A similarity was however, observed between ClO\(_4^-\) \( \Delta^{17}\text{O} \) values and ClO\(_3^-\) \( \Delta^{17}\text{O} \) values with respect to oxidation of Cl in precursor species and final ClO\(_3^-\)/ClO\(_4^-\) ratios. Both
final ClO₃⁻/ClO₄⁻ ratios and Δ¹⁷O values of ClO₄⁻ and ClO₃⁻ were highest for the O₃ Cl⁻(aq) experiments, followed by those of the O₃ OCl⁻ experiments, and lastly the O₃ ClO₂⁻ experiments. This further suggests a model in which pathways with common initial reaction stages for ClO₃⁻ and ClO₄⁻ production exist.

2.5.4.8 Isotopic Characteristics of ClO₄⁻ and their Connection to Formation Pathways

The isotopic composition of precursor compounds involved in ClO₄⁻ formation via UV and O₃ processes and/or fractionation caused by isotope exchange reactions or kinetic processes could be used to explain our ClO₄⁻ isotope values. Isotope exchange involves reversible equilibrium reactions in which products can become either heavier or lighter than the original reactants, whereas kinetic processes are irreversible and generally result in products enriched in the lighter isotopes. The oxidation state of a species can also affect equilibrium reactions, with species having higher oxidation states often enriched in the heavier isotope (Kendall et al., 1998, 2008; Schauble et al., 2003). The δ³⁷Cl values of ClO₄⁻ formed from UV and O₃ oxidation processes indicate that the heavier ³⁷Cl isotope was preferentially accumulated in ClO₄⁻ compared to initial species and other final products, with the exception of two experiments (O₃ oxidation of dry Cl⁻ and OCl⁻). These results could be indicative of equilibrium processes resulting in branching fractionation of intermediate species leading to accumulation of the heavier Cl isotope in the species with the higher oxidation state (ClO₄⁻). The two exceptions (O₃ oxidation of dry Cl⁻ and OCl⁻) may be indicative of kinetic isotope effects that cause enrichments of the heavier isotopes in the residual reactants. The difference in isotope compositions of ClO₄⁻ produced by O₃ oxidation of dry Cl⁻ versus those of aqueous Cl⁻ suggests that reactions involving ClOₓ oxidation of solid compounds may not fractionate isotopes in the same way as ClOₓ (aq) species. It is possible that one may be kinetically based whereas the other might be equilibrium based. Δ¹⁷O values of ClO₄⁻ also suggest that ClO₄⁻ may be formed by more than one pathway and its isotopic composition may be the result of a mixture of the compositions of ClO₄⁻ formed by each distinct pathway.

2.5.4.9 Implications of ClO₄⁻ Δ¹⁷O values in O₃ Experiments

Conclusions regarding the mechanisms responsible for the ClO₄⁻ isotopic compositions observed in our study were made by considering proposed alternative O₃-ClOₓ oxidation reactions and evaluating them with respect to which ClO₄⁻ formation pathways are plausible based on the Δ¹⁷O values of the ClO₄⁻ produced in our O₃ experiments.

O₃-Dry Chloride

It is difficult to evaluate the formation of ClO₄⁻ from O₃ oxidation of dry Cl⁻ as it is uncertain if reactions take place on the surface of the salt, on the glass surface, or in the gas phase; nor if water vapor in the O₂ cylinders or bound water on the solids plays a role in reactions leading to its formation.

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The O₃ dry Cl⁻ experiment was the only experiment that resulted in a ClO₃⁻/ClO₄⁻ product molar ratio (0.18) less than 1. The O₃ oxidation of dry Cl⁻ produced ClO₄⁻ with the highest Δ¹⁷O values (+33 ‰), but the values were only slightly higher than ClO₄⁻ produced by O₃ oxidation of aqueous Cl⁻ (aq) (+29 ‰ to +32 ‰). This suggests that not only does liquid H₂O not need to be present for the formation of ClO₄⁻ to occur but that all four O in ClO₄⁻ are sourced from O₃, as O₃ produced from the O₃ generator had a bulk Δ¹⁷O value of +24.5 ‰.

From the proposed O₃-ClOₓ oxidation reactions (Table 2.5.4), four pathways with different initial reaction sequences (Rxn’s 1–3; Rxn’s 1, 8 – 9; Rxn’s 1, 8, and 10–11; and Rxn’s 1, 3, 8, and 13) but with the same final reaction sequence (Rxn’s 4 and 12) were identified that could produce ClO₄⁻ with four O from O₃ in the absence of water (Table 2.5.4; Figure 2.5.16). Due to missing reaction rates, little can be inferred regarding the ClO₄⁻ pathways proposed. Aside from ClO₄⁻, the only other detectible product was ClO₃⁻ for which we were able to identify at least one pathway that formed ClO₃⁻ without reactions involving H₂O (Figure 2.5.17). This pathway has the same initial reaction sequences as formation of ClO₄⁻ but a different final reaction (Table 2.5.4; Rxn 29). Due to the low mass of ClO₃⁻ produced, no ClO₃⁻ Δ¹⁷O values are available.

There has been much debate regarding the position (central vs terminal O atom) of the Δ¹⁷O anomaly in the O₃ molecule and whether it is preferentially transferred to other molecules during oxidation reactions (Berhanu et al., 2012; Bhattacharya et al., 2008; Lyons et al., 2001). Our ClO₄⁻ Δ¹⁷O values (+29 ‰ to +32 ‰) being higher than those of the generated O₃ (+24.5 ‰) support the theory that terminal O atoms in O₃ are preferentially enriched in the Δ¹⁷O anomaly and that the terminal O atoms carrying the anomaly are preferentially incorporated into the ClOₓ compounds. If we assume that site preference is on the terminal O atom and that the Δ¹⁷O of the terminal O atom is transferred to ClO₄⁻, then we could theoretically calculate the Δ¹⁷O values of ClO₄⁻ that we would expect if there was complete or partial preference of the heavy isotope on the terminal O atom of O₃ using Equation 5 (Bhattacharya et al., 2008; Lyons et al., 2001). Application of Equation 5 and assuming complete terminal O site preference would result in a ClO₄⁻ with a maximum Δ¹⁷O value of +37 ‰. With no terminal site preference, the maximum Δ¹⁷O of ClO₄⁻ would be +24.5 ‰. Our ClO₄⁻ Δ¹⁷O values (+29 ‰ to +32 ‰) were between these two values, indicating there is at least some preferential incorporation of terminal O atoms from O₃ into ClO₄⁻.

\[
\Delta^{17}O \text{ of terminal O}_3 \text{ atoms} = \frac{3}{2} \times \Delta^{17}O \text{ of bulk O}_3
\]  (5)
However, it is not possible to completely constrain site preference because it is also possible that the observed $\Delta^{17}O$ value of ClO$_4^-$ from O$_3$ oxidation of dry Cl$^-$ is less than the value for complete preference due to the addition of a small amount of ClO$_4^-$ formed from reaction pathways incorporating three or less O from O$_3$ or partial O exchange during the reactions.
Table 2.5.4. Proposed reactions possibly leading to the formation of ClO₄⁻ via O₃ oxidation of ClOₓ species.

<table>
<thead>
<tr>
<th>Reaction #</th>
<th>Reaction</th>
<th>Reaction Rate</th>
<th>Method</th>
<th>Comments</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(O_3 + Cl^- \rightarrow OCl^-/HOCl + Cl + O_2)</td>
<td>Based on O₃(aq) depletion</td>
<td>pH &gt; 3</td>
<td>Hoigné et al. (1985) Levanov et al. (2003)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(O_3 + OCl^- \rightarrow O_2 + ClO_2^-)</td>
<td>K=30 M⁻¹s⁻¹ (1985; 1996)</td>
<td>Based on O₃(aq) depletion</td>
<td>Hoigné et al. (1985) Siddiqui (1996)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(O_3 + ClO_2^- \leftrightarrow O_2 + ClO)</td>
<td>²Kₑ=8.2(4) x 10⁶ M⁻¹s⁻¹ (2002) ²Kₑ=1.8 x 10⁴ M⁻¹s⁻¹ (1984) ²Kₑ=(4±1) x 10⁶ M⁻¹s⁻¹ (1985)</td>
<td>Determined under pseudo-first order conditions using stopped flow spectrometry Pulse Radiolysis Pulse Radiolysis and Stopped Flow Spectrometry</td>
<td>Nicoson et al. (2002) Klänning et al. (1985) Bühler et al. (1984)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>(ClO_2 + O_3 \rightarrow ClO_3 + O_2)</td>
<td>K=(1.05 ± 0.10)X10³ M⁻¹s⁻¹ (1985)</td>
<td>Stopped flow spectroscopy of ClO₃(aq) and O(aq)</td>
<td>Klänning et al. (1985)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemical Reaction</td>
<td>Kinetic Constant or Reaction Condition</td>
<td>Notes or References</td>
<td></td>
<td></td>
</tr>
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<td>---</td>
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<td>----------------------------------------</td>
<td>---------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>$O_2ClOClO_3 + O_3 \rightarrow Cl_2O_7 + O_2$</td>
<td></td>
<td>Sander et al. (1989) Wiberg et al. (2001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$Cl_2O_7 + H_2O \rightarrow 2ClO_4^- + 2H^+$</td>
<td></td>
<td>Wiberg et al. (2001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>$OH + HOCl \rightarrow OCl + H_2O$</td>
<td>$K = (1.4 \pm 0.1) \times 10^8$ M$^{-1}$s$^{-1}$ (1997)</td>
<td>Pulse Radiolysis of HOCl and H$_2$SO$_4$ Zuo et al. (1997)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>$ClO + O_3 \rightarrow ClOO + O_2$</td>
<td></td>
<td>Vaida and Simon (1995)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>$ClO + ClO \rightarrow Cl + ClOO$ OR $ClO + ClO \rightarrow Cl + OCIO$</td>
<td></td>
<td>Sander et al. (1989)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>$Cl + O_3 \rightarrow ClO + O_2$</td>
<td>Flash Photolysis wavelength &gt; 300 nm</td>
<td>Sander et al. (1989)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>$ClO_3 + HO \rightarrow HClO_4$</td>
<td>Boron Doped Diamond Film Electrodes, speculated to occur in the stratosphere</td>
<td>Considered Hubler et al. (2014) Simonatis and Heicklen (1975)</td>
<td></td>
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</tr>
<tr>
<td>13</td>
<td>$OCIO + HO \rightarrow HClO_2$</td>
<td>Boron Doped Diamond Film Electrodes</td>
<td>Considered Hubler et al. (2014)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>$O_2ClOClO_3 + H_2O \rightarrow ClO_5^- + ClO_2^- + 2H^+$</td>
<td>$K = 180$ M$^{-1}$s$^{-1}$</td>
<td>Assumed attack of water on the more acidic chlorine Adopted from previous studies Quiroga and Perissinotti (2005) Wiberg et al. (2001)</td>
<td></td>
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<tr>
<td></td>
<td>Reaction</td>
<td>Assumed diffusion control by using the expression of Smoluchoswski and the Stokes-Einstein Equation</td>
<td>Estimated/Proposed</td>
<td>Reference(s)</td>
<td></td>
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<td>-------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>$OCIO + ClO \rightarrow ClOClO_2$</td>
<td>$K = 7.4 \times 10^9 \text{M}^{-1}\text{s}^{-1}$</td>
<td>Estimated</td>
<td>Quiroga and Perissinotti (2005) Sander et al. (1989)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>$Cl_2O_4 + O_3 \rightarrow Cl_2O_4$</td>
<td></td>
<td>Hypothesised</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>$Cl_2O_4 + H_2O \rightarrow HClO + ClO_4^- + H^+$</td>
<td>$K = 180 \text{M}^{-1}\text{s}^{-1}$</td>
<td>Estimated</td>
<td>Quiroga and Perissinotti (2005) Wiberg et al. (2001)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>$OCIO + Cl \rightarrow ClClO_2$</td>
<td>$K = 7.8 \times 10^9 \text{M}^{-1}\text{s}^{-1}$</td>
<td>Estimated</td>
<td>Quiroga and Perissinotti (2005)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>$Cl_2O_2 + O_3 \rightarrow Cl_2O_3$</td>
<td></td>
<td>Hypothesised</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>$OCIO + ClO_3 \rightarrow O_2ClOClO_2$</td>
<td>$K = 7.5 \times 10^9 \text{M}^{-1}\text{s}^{-1}$</td>
<td>Estimated</td>
<td>Quiroga and Perissinotti (2005)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>$O_2ClOClO_2 + H_2O \rightarrow 2ClO_3^- + 2H^+$</td>
<td>Assumed attack of water on the more acidic chlorine</td>
<td>Adopted from</td>
<td>Quiroga and Perissinotti (2005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$O_2ClOClO + H_2O \rightarrow ClO_3^- + ClO_4^- + 2H^+$</td>
<td>$K_{ClO_3^-} = 180 \text{M}^{-1}\text{s}^{-1}$, $K_{ClO_4^-} = ?$</td>
<td>previous studies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.5.16. Combined final pathways determined responsible for ClO₄⁻ formation by O₃ oxidation of Cl⁻ (dry and aq). Note the thick forest dark red lines indicate the initial reactions that some of the species may share while the dark blue lines indicate the final reactions all pathways share. All other colored lines (green, pink, aqua, and blue) represent reactions unique to each of the four proposed pathways. All four pathways lead to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure 2.5.17. Combined final pathways determined responsible for ClO$_4^-$ formation by O$_3$ oxidation of Cl$^-$ including possible pathways for ClO$_3^-$ formation (aq). Note the thick dashed lines indicate the initial reactions that are possibly shared between ClO$_4^-$ and ClO$_3^-$ pathways and the thick aqua lines represent the final reactions leading to ClO$_3^-$ formation. Both ClO$_3^-$ pathways lead to the formation of one ClO$_3^-$ molecule containing one O from O$_3$. 
O₃-Aqueous Chloride


Our ClO₄⁻Δ¹⁷O values (+29 ‰ to +32 ‰) suggest that the O₃ oxidation of Cl⁻ (aq) includes intermediate reactions that will lead to the formation of a ClO₄⁻ molecule with all or nearly all (depending on the final site preference value) O originating from O₃; therefore, any mechanisms resulting in a ClO₄⁻ with less than four O from O₃ were dismissed as plausible pathways (Appendix A: Figures A.9 – A.26). Excluding the pathways that do not result in transfer of four O from O₃ lowers the number of possible mechanisms from twenty-six to only eight, all of which require the formation of both ClO₂ (aq) and the ClO₃ radical (Appendix A: Figures A.1 – A.8).

Of the eight remaining pathways (Appendix: Figures A.1 – A.8), four of them (Appendix A Figures A.1 – A.4) require the reaction of the ClO₃ radical with an OH radical and four of them (Appendix A: Figures A.5 – A.8) require the formation of the Cl₂O₇ species. The Cl₂O₇ species hydrolyzes to form two ClO₄⁻ molecules (Table 2.5.4; Rxn 7), one containing three and the other four O from O₃. It has been speculated that Cl₂O₇ is involved in the production of ClO₄⁻, but our ClO₄⁻Δ¹⁷O values indicate that this may not be the case as only one of the two ClO₄⁻ molecules formed from hydrolysis of this intermediate species has a structure in which all O’s originate from O₃. However, the unknown degree of site preference can allow for a limited amount of ClO₄⁻ with only three O from O₃ and thus it is possible that a mixture of the two ClO₄⁻ molecules produced from hydrolysis of the Cl₂O₇ species is in part responsible for the ClO₄⁻ formed in our Cl⁻ (aq) experiments. It might be unlikely that reactions involving the Cl₂O₇ species are responsible for substantial ClO₄⁻ production, unless unknown Cl₂O₇ reaction pathways exist that include transfer of an additional O from O₃ (e.g. O radical) rather than from water. However, given the high Δ¹⁷O value of ClO₄⁻ observed, it is possible that ClO₄⁻ is a result of mixing between branching pathways involving the OH radical and Cl₂O₇ species.

Elimination of the pathways involving the Cl₂O₇ species would reduce the number of plausible mechanisms responsible for ClO₄⁻ formation by O₃ oxidation of Cl⁻ (aq) to only four, all of which go through the same final reaction sequence (Table 2.5.4; Rxn’s 4 and 12) involving the formation of a ClO₃ radical that is oxidized by an OH radical (simultaneously represented by Figure 2.5.16). Currently it is unclear as to whether the O in the OH radical originates from O₃ or has another origin. Our interpretation of the ClO₄⁻ Δ¹⁷O values along with the evaluation of the proposed pathways suggest that ClO₄⁻ with four O from O₃ is possibly mainly formed through a final oxidation reaction involving an OH radical and not through the Cl₂O₇...
intermediate species, as suggested by others. If our analysis is correct then it would mean that the O in the OH radical most likely originates from O₃.

The Δ¹⁷O values of ClO₃⁻ produced during ClO₄⁻ production from O₃ oxidation of Cl⁻ (aq) indicate that only one O in ClO₃⁻ could be derived from an O₃ molecule. This could occur through reactions that lead to the incorporation of a single O from O₃ or through a mixture of pathways that lead to zero to four O being incorporated from O₃. If ClO₄⁻ and ClO₅⁻ were formed from the same initial reactions (Rxn’s 1– 3; Rxn’s 1, and 8 – 9; Rxn’s 1, 8, and 10 – 11; and Rxn’s 1, 3, 8, and 13); they alone would contribute at least two O from O₃ to the ClO₅⁻ molecules, with the final ClO₅⁻ containing anywhere from two to three O from O₃. Even pathways only sharing two of the initial reactions (Rxn’s 1 and 8) would result in a ClO₃⁻ with two O from O₃ (Figure 2.5.18). In addition, none of the pathways that we identified as possible ClO₄⁻ formation mechanisms (Figure 2.5.16) produced ClO₅⁻ directly from the production of ClO₄⁻, further indicating the independence of ClO₄⁻ from ClO₃⁻ pathways. A couple of factors could be responsible for ClO₅⁻ only having one O from O₃ including: (1) non O₃ related reactions that have not yet been identified and (2) oxygen exchange during equilibrium reactions. In support of the likelihood of independent ClO₅⁻ production pathways, ClO₃⁻/ClO₄⁻ molar ratios (3000-4400) are orders of magnitude higher than for dry oxidation of Cl⁻ even though ClO₄⁻ production was relatively similar. Based on the ratios we would expect that the major reactions lead to ClO₅⁻ formation and if so, based on our ClO₃⁻ Δ¹⁷O values, we could speculate that the major source of oxidation for formation of ClO₅⁻ is not O₃, but rather another species. Given our relatively high ClO₃⁻/ClO₄⁻ ratios in our Cl⁻ (aq) experiments compared to those of O₃ oxidation of dry Cl⁻, it is evident that hydrolysis reactions are not as important for ClO₄⁻ formation as they are for ClO₃⁻. Our ClO₃⁻/ClO₄⁻ ratios in conjunction with our ClO₄⁻ Δ¹⁷O values further suggest and support our theory that the involvement of O₃ in ClO₄⁻ formation mechanisms due to oxidation of Cl⁻ is not hindered by the presence of H₂O, but rather that H₂O may not be required for formation of ClO₄⁻ to occur.

It may be possible to determine the feasibility of the ClO₄⁻ and ClO₅⁻ pathways by evaluating the reaction rates involved, but not all reactions have reported reaction rates (Table 2.5.4). One key step in which reaction rates are known is the decomposition of the OCI⁻ species that when oxidized can either form ClO₂⁻ or the OCl radical (Table 2.5.4; Rxn’s 2 and 8, respectively). The reaction rates for these two oxidation reactions indicate that the faster reaction leads to the formation of the OCl radical by means of an OH radical, whereas the slower reaction leads to formation of ClO₂⁻ by means of the O₃ molecule (Rxn’s 8 and 2, respectively). Compared to other ClOₓ species, Cl⁻ (dry and aqueous) reacted slowly and O₃ was unlimited, giving way for more reactions with O₃ to occur. Reactions 2 and 8 (Table 2.5.4) both imply that O₃ reactions occur much slower than reactions involving a non O₃ species and thus because major reactions are expected to form ClO₃⁻, it is likely that pathways leading to ClO₃⁻ follow Reaction 8 (Table
2.5.4), which involves the OH radical, and pathways leading to ClO₄⁻ follow Reaction 2 (Table 2.5.4), which involves the O₃ molecule. The reactions for the decomposition of the ClO₂ species also support ClO₄⁻ being formed by slow reactions involving O₃. In the ClO₄⁻ pathways identified (Figure 2.5.16), we propose that ClO₂ is oxidized to the ClO₃ radical by O₃ (Table 2.5.4; Rxn 4). This reaction is much slower compared to reactions that either lead to the formation of the Cl₂O₂ species (Table 2.5.4; Rxn 18), the formation of ClO₃⁻ (Table 2.5.4; Rxn 29), or the formation of the Cl₂O₃ species, indicating that the pathways we propose may well be the pathways responsible for ClO₄⁻ formation in this study.
Figure 2.5.18. Combined final pathways determined responsible for ClO$_4^-$ formation by O$_3$ oxidation of Cl$^-$ (aq) including possible pathways for ClO$_5^-$ formation. Note the thick dashed lines indicate the initial reactions that are possibly shared between ClO$_4^-$ and ClO$_5^-$ pathways and the thick aqua lines represent the final reactions leading to ClO$_5^-$ formation. Both ClO$_5^-$ pathways lead to the formation of one ClO$_5^-$ molecule containing one O from O$_3$. 
With the exception of reaction 1 (Table 2.5.4), all mechanisms possibly leading to ClO$_4^-$ production from O$_3$ oxidation of OCl$^-$ (aq) (Appendix: Figures A.27 – A.49) were the same as the mechanisms identified leading to ClO$_4^-$ formation from O$_3$ oxidation of Cl$^-$ (aq). For further evaluation, these twenty-six mechanisms were divided into five groups: [1] pathways leading to ClO$_4^-$ with only three O atoms from O$_3$ (Appendix: Figures A.27 – A.30), [2] pathways leading to production of two ClO$_4^-$ molecules containing two and three O atoms from O$_3$, respectively (Appendix: Figures A.31 – A.34), [3] pathways leading to ClO$_4^-$ with only two O atoms from O$_3$ (Appendix: Figures A.35 – A.39), [4] pathways involving the formation of the Cl$_2$O$_5$ intermediate species (Appendix: Figures A.40 – A.43), and [5] pathways involving reactions 16 and 19, reactions involving the oxidation of Cl$_2$O$_3$ into Cl$_2$O$_4$ and oxidation of Cl$_2$O$_2$ into Cl$_2$O$_3$ by O$_3$, respectively (Appendix: Figures A.44 – A.49). Assuming that the $\Delta^{17}$O values (+29‰ to +33‰) of ClO$_4^-$ from the O$_3$-Cl$^-$ (dry and aqueous) experiments can be used as a reference to determine site preference and reaction selectivity of terminal O atoms from O$_3$ into ClO$_4^-$ we would expect that each O from O$_3$ would contribute about 8‰. Based on this assumption, our ClO$_4^-$-$\Delta^{17}$O values produced from OCl$^-$ (+16‰ to +20‰) suggest that the O$_3$ oxidation of OCl$^-$ (aq) results in a ClO$_4^-$ molecule with at least two but less than three O from O$_3$, thus we were unable to eliminate pathways based on the number of O atoms derived from O$_3$ as the $\Delta^{17}$O values of our ClO$_4^-$ could be the result of a combination of pathways yielding ClO$_4^-$ having zero to four O from O$_3$. Furthermore, OCl$^-$ is a direct product of the reaction between Cl$^-$ and O$_3$ (Table 2.5.4: Rxn 1), thus it is also possible that OCl$^-$ and Cl$^-$ share the same pathway leading to the production of ClO$_4^-$.

Therefore, reductions in pathways were made by eliminating pathways with reactions and species that have not been observed or discussed in detail in literature.

The Cl$_2$O$_5$ species has two possible isomers (Table 2.5.4) and only one isomer will lead to the formation of ClO$_4^-$ (Quiroga et al., 2005). Due to the lack of knowledge regarding the isomer structures of Cl$_2$O$_5$ and the likeliness of a mechanism forming ClO$_3^-$ from the Cl$_2$O$_5$ species instead, all mechanisms involving Cl$_2$O$_5$ were regarded as being unlikely to account for ClO$_4^-$ formed in our study (Appendix: Figures A.40 – A.43) (Quiroga et al., 2005). In addition, all pathways involving reactions 16 and 19, the formation of ClO$_4^-$ from the reaction between OCl and ClO$_3$ radicals, were also eliminated as these reactions have not been demonstrated to exist and were only speculated to occur (Appendix: Figures A.44 – A.49). Exclusion of these pathways (Appendix: Figures A.40 – A.49) resulted in thirteen mechanisms (Appendix: Figures A.27 – A.39) capable of forming the ClO$_4^-$ in our study, nine more than those identified for ClO$_4^-$ formed from the O$_3$ oxidation of dry Cl$^-$ (simultaneously represented in Figure 2.5.16). Similarly to O$_3$ oxidation of Cl$^-$ (aq) the remaining pathways all require the formation of ClO$_2$ (aq) and a subsequent ClO$_3$ radical (simultaneously represented in Figure 2.5.19).
We could hypothetically theorize which pathways might be more important than others based on the rate of reaction of Cl\(^-\) versus that of OCl\(^-\) in the presence of O\(_3\). Complete decay of OCl\(^-\) happened in hours compared to Cl\(^-\) loss which took months and did not fully complete. Molar yields of ClO\(_4^-\) for the O\(_3\)-OCl\(^-\) (0.01-0.07 \%) experiments were at least an order of magnitude higher than the molar yields of ClO\(_4^-\) from the O\(_3\)-Cl\(^-\) (aq) experiments (<0.001 \%) even though the initial mass of Cl\(^-\) was two to three orders of magnitude higher than the initial molar mass of OCl\(^-\) (Table 2.5.3). Although the molar mass of transformed Cl\(^-\) was less (1.5 \%) compared to transformed OCl\(^-\) (99.98 \%), in the Cl\(^-\) experiments there was an excess amount of Cl\(^-\) and O\(_3\) in solution as O\(_3\) was being continuously input into the system, allowing for continuous reactions between Cl\(^-\) and O\(_3\) to occur. Yet the rate of reaction between Cl\(^-\) and O\(_3\) might have been much slower compared to the rate of reactions between Cl\(^-\) and other species. Due to the rate of reaction and input rate of O\(_3\) for the oxidation of OCl\(^-\), O\(_3\) was likely limiting, perhaps facilitating reactions not involving O\(_3\) and thus allowing alternate reactions (e.g. Rxn’s 7, 14, 22, and 17) not observed in O\(_3\) oxidation of Cl\(^-\) to occur (Figure 2.5.19; Table 2.5.4). Some of these reactions (Table 2.5.4; Rxn’s 7, 14, and 7) are hydrolysis reactions, reactions which, as previously suggested, are not considered necessary for ClO\(_4^-\) to form from O\(_3\) oxidation of Cl\(^-\) (aq). Based on this data, it is assumed that ClO\(_4^-\) production from O\(_3\) oxidation of OCl\(^-\) is not entirely dependent on the same pathway as that of ClO\(_4^-\) from O\(_3\) oxidation of Cl\(^-\), but is rather a mixture of that pathway and other pathways not entirely dependent on O\(_3\) or O\(_3\) related oxidant reactions.
Figure 2.5.19. Combined final pathways determined responsible for ClO$_4^-$ formation by O$_3$ oxidation of OCl$^-$ (aq). Note the thick forest dark red lines indicate the reactions that some of the species may share while other colored lines represent reactions unique to each of the proposed pathways. All pathways lead to the formation of a ClO$_4^-$ molecule containing either two or three O’s from O$_3$. 
Our results for the OCl\(^-\) experiments further support the theory that formation of ClO\(_3^-\) may be independent of formation of ClO\(_4^-\). \(\Delta^{17}O\) values for ClO\(_3^-\) (-0.02 \%o to + 1.37 \%o) indicate that the O in ClO\(_3^-\) is not primarily derived from O\(_3\) (+ 24.5 \%o). Out of the thirteen possible pathways (Appendix: Figures A.27 – A.39), only two pathways (Appendix A: Figures A.35 – A.38) result in the formation of a ClO\(_3^-\) molecule along with ClO\(_4^-\). These pathways would produce a ClO\(_3^-\) with two O atoms derived from O\(_3\) and thus, cannot be responsible for ClO\(_3^-\) formation in our study. Three pathways (Figure 2.5.20), independent of ClO\(_4^-\) formation, were determined to be possible for formation of a ClO\(_3^-\) molecule with no O from O\(_3\). Given that reaction sequences that would form ClO\(_3^-\) during O\(_3\) oxidation of Cl\(^-\) would form ClO\(_3^-\) with two O from O\(_3\) (Figure 2.5.18), the ClO\(_3^-\) formed from O\(_3\) oxidation of OCl\(^-\) is consistent as reaction 1 (Table 2.5.4) would be eliminated reducing the contribution of O from O\(_3\) to near zero.

Final ClO\(_3^-\)/ClO\(_4^-\) product ratios for O\(_3\) oxidation of OCl\(^-\) (aq) (482-527) were not as high as those for O\(_3\) oxidation of Cl\(^-\) (aq). These molar ratios indicate a higher production of ClO\(_3^-\) compared to ClO\(_4^-\), supporting our hypothesis that ClO\(_4^-\) formation by O\(_3\) oxidation of OCl\(^-\) may be independent of that of ClO\(_3^-\). However, our product yields indicate that a significant amount of OCl\(^-\) is transformed to Cl\(^-\), not ClO\(_3^-\) or ClO\(_4^-\), suggesting that the main pathway rather leads to Cl\(^-\) not ClO\(_3^-\) (Table 2.5.3). This pathway may be the result of reaction 1 (Table 2.5.4), a back reaction involving the conversion of OCl\(^-\) to Cl\(^-\).
Figure 2.5.20. Combined final pathways determined responsible for \( \text{ClO}_4^- \) formation by \( \text{O}_3 \) oxidation of \( \text{OCl}^- \) including possible pathways for \( \text{ClO}_3^- \) formation (aq). Note the thick dashed lines indicate the initial reactions that are possibly shared between \( \text{ClO}_4^- \) and \( \text{ClO}_3^- \) pathways and the thick aqua lines represent the final reactions leading to \( \text{ClO}_3^- \) formation.
**O₃-Chlorite**

ClO₄⁻ produced from O₃ oxidation of ClO₂⁻ (Δ¹⁷O = +12 ‰ to +13 ‰) had a lower Δ¹⁷O than ClO₄⁻ produced from OCl⁻ or Cl⁻. Using the Δ¹⁷O values for ClO₄⁻ produced from O₃ oxidation of Cl⁻ (aq) (+29‰ to +32‰), for which all four O must come from O₃, as a reasonable approximation of the site preference value, we would expect that ClO₄⁻ incorporating two O from O₃ would have a Δ¹⁷O value near the ranges of +14.5 ‰ to +16‰. This suggests that O₃ oxidation of ClO₂⁻ results in the transfer of two or less O from O₃.

A total of six mechanisms were postulated to form ClO₄⁻ from O₃ oxidation of ClO₂⁻ (aq) (Appendix A: Figures A.50 – A.55). All pathways starting from ClO₂⁻ produce ClO₄⁻ with one or two O from O₃. Two pathways (Appendix A: Figures A.54 and A.55) were eliminated because they either required formation of the Cl₂O₅ species or the formation of the Cl₂O₄ species by the oxidation of Cl₂O₃ by O₃ (Rxn 16; Table 2.5.4), a reaction we speculated could occur but has not been documented, leaving only four (Appendix: Figures A.50 – A.53) possible pathways (simultaneously represented in Figure 2.5.21). Two (Appendix A: Figures A.52 – A.53) of the four pathways produce a ClO₄⁻ with one O from O₃ and two (Appendix A: Figures A.50 – A.51) produce a ClO₄⁻ with two O’s from O₃ (similar to that indicated by the Δ¹⁷O values of ClO₄⁻).

The pathways (Appendix A: Figures A.50 – A.51) leading to ClO₄⁻ with two O from O₃ are consistent with the pathways observed for O₃ oxidation of Cl⁻ (aq) and OCl⁻ given the consistent decrease in Δ¹⁷O of ClO₄⁻ indicating a loss of O transfer from each reaction step starting with O₃ oxidation of Cl⁻ (aq). In the Cl⁻ (aq) pathway, the first reaction donates an O to Cl⁻ from O₃ (Rxn 1; Table 2.5.4) and any subsequent reactions (Rxn 2 or Rxn’s 8 and 13; Table 2.5.4) add an additional O, derived from O₃, to OCl⁻ to form ClO₂⁻.
Figure 2.5.21. Combined final pathways determined responsible for ClO$_4^-$ formation by O$_3$ oxidation of ClO$_2^-$ (aq). Note the thick forest dark red lines indicate the reactions that some of the species may share while other colored lines represent reactions unique to each of the proposed pathways. All pathways lead to the formation of a ClO$_4^-$ molecule containing either one or two O’s from O$_3$. 
ClO₃⁻ was the major product of the O₃-ClO₂⁻ experiments and in accordance with the previous ClO₃⁻-Δ¹⁷O values of the Cl⁻ (aq) and OCl⁻ (aq) experiments, Δ¹⁷O values (+3‰ to +4‰) of ClO₃⁻ formed from O₃ oxidation of ClO₂⁻ suggest that ClO₃⁻ formation pathways may be independent of those of ClO₄⁻ as only about half of one O in ClO₃⁻ is derived from O₃. This is further supported by the ClO₃⁻/ClO₄⁻ molar ratios (902 – 1111) which indicate that the main pathway would lead to ClO₃⁻ formation and the minor pathways to ClO₄⁻. Four pathways (Figure 2.5.22) were proposed as possibly leading to the ClO₃⁻ produced in our ClO₂⁻ (aq) experiments. Three pathways share the initial reaction (Reaction 3: formation of ClO₂ (aq)) with the ClO₄⁻ pathways, but then diverge into separate reaction sequences to produce ClO₃⁻ (Figure 2.5.22). All reactions proposed to occur after reaction 3 that lead to ClO₃⁻ formation are fast (Rxn’s 15 and 29; Table 3.4) compared to reaction 4, which leads to ClO₄⁻ formation (Figure 2.5.22; Table 2.5.4), indicating that our proposed mechanisms for ClO₄⁻ and ClO₃⁻ formation might be correct. Out of the four ClO₃⁻ pathways proposed, two of the pathways (those involving Rxn’s 26 and 27; Table 2.5.4 and Figure 2.5.22) form a ClO₃⁻ molecule with one O from O₃ and the other two pathways (those involving Rxn’s 20 – 21 and 29; Table 2.5.4; Figure 2.5.22) form a ClO₃⁻ molecule with possibly zero O from O₃ depending on where the O from O₃ is located in the Cl₂O₅ structure and whether the O in the OH radical is derived from the O₃ molecule. These pathways suggest that ClO₃⁻ formed from O₃ oxidation of ClO₂⁻ may be the result of a mixture of different formation mechanisms (Table 2.5.4).
Figure 2.5.22. Combined final pathways determined responsible for ClO$_4^-$ formation by O$_3$ oxidation of ClO$_2^-$ including possible pathways for ClO$_3^-$ formation (aq). Note the thick dashed lines indicate the initial reactions that are possibly shared between ClO$_4^-$ and ClO$_3^-$ pathways and the thick aqua lines represent the final reactions leading to ClO$_3^-$ formation.
**O₃-Chlorine Dioxide**

The proposed mechanisms for the formation of ClO₄⁻ from molecular O₃ and ClO₂ (aq) are summarized in Appendix A Figures A.56 – A.61 and either produce a ClO₄⁻ with one or two O from O₃. ClO₄⁻ produced from O₃ oxidation of ClO₂ (aq) had Δ¹⁷O values (+16 ‰ to +17 ‰) similar, yet slightly less than oxidation of OCl⁻ and higher than oxidation of ClO₂⁻. Assuming our estimated value for the site preference are correct based on ClO₄⁻ produced from O₃ oxidation of Cl⁻ (e.g. O ≈ 8 ‰), all pathways producing a ClO₄⁻ with less than two O from O₃ were eliminated as possible ClO₄⁻ formation pathways (Appendix A: Figures A.57 – A.61), thus resulting in only one possible pathway (Figure 2.5.23), consistent with the most likely pathways described previously.

A reason for the slightly higher Δ¹⁷O value for ClO₄⁻ produced from ClO₂ (aq) compared to ClO₂⁻ is unclear but perhaps is due to variation in the site preference value. The transformation of ClO₂⁻ to ClO₂ (aq) does not involve an O transfer. Perhaps the much higher concentration (60 – 69 mmol) of reactant in the ClO₂⁻ oxidation experiment compared to ClO₂ (aq) (0.5 – 1.1 mmol) experiment produced an effect on the site preference value. The oxidation of ClO₂⁻ (rapid reaction) may have resulted in O₃ limitations, while for ClO₂ (aq) oxidation (also rapid) O₃ may not have been limiting due to the lower reactant concentration. The yield of ClO₄⁻ for both ClO₂ (aq) and ClO₂⁻ were the highest for all experiments although they were twenty to thirty fold higher for ClO₂ (aq) than ClO₂⁻.

The ClO₃⁻/ClO₄⁻ molar ratios (21-30) for O₃ oxidation of ClO₂ (aq) were lower compared to those of the ClO₂⁻ (aq) and even the Cl⁻ (aq) (3022 – 4431) and OCl⁻ (482 – 569) experiments, supporting the previous postulations that ClO₃⁻ production is due to reactions unrelated to ClO₄⁻ production and that major ClO₃⁻ pathways may diverge from those of ClO₄⁻ early in the reaction sequence. Aside from ClO₄⁻, ClO₃⁻ was the only other species produced in the ClO₂ (aq) experiments, but without isotopic data for ClO₃⁻, we were unable to determine the mechanisms involved in ClO₃⁻ formation.
Figure 2.5.23. O₃ oxidation of ClO₂ (aq). Note the thick maroon lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 2.5.4. This pathway leads to the formation one ClO₄⁻ molecule containing two O from O₃.
2.5.4.10 Overall Conclusions Based on $\Delta^{17}$O of O$_3$ Experiments

The $\Delta^{17}$O values of ClO$_4^-$ produced by O$_3$ oxidation of ClO$_x$ species provide a better understanding of the role the O$_3$ molecule plays in the transfer of O atoms into produced ClO$_4^-$ as well as help to identify key reactions involved in ClO$_4^-$ formation. There was a consistent decrease in O atoms incorporated from O$_3$ into ClO$_4^-$ with increasing oxidation state of Cl as the reactant species, with the exception of ClO$_2$ (aq). The ClO$_4^-$ pathways proposed for O$_3$ oxidation of Cl$^-$, OCl$^-$, and ClO$_2^-$ are consistent with these observations as they account for the loss of O$_3$ O atoms through each initial reaction step: Cl$^-$ to OCl$^-$ (Rxn 1; Table 2.5.4) and OCl$^-$ to ClO$_2^-$ (Rxn 2; Table 2.5.4). Moreover, regardless of the reactant species (Cl$^-$, OCl$^-$, ClO$_2^-$, or ClO$_2$ (aq)) all ClO$_4^-$ pathways include the ClO$_2$ and the ClO$_3$ radical. Because ClO$_2$ (aq) is a required intermediate, the generation pathway must include Reactions 4 (Table 2.5.4), the oxidation of ClO$_2$ to ClO$_3$.

The $\Delta^{17}$O values of ClO$_3^-$ compared to those of ClO$_4^-$ also indicate that ClO$_4^-$ and ClO$_3^-$ are formed through independent pathways. ClO$_3^-$ was the main species produced, indicating that major reactions of O$_3$ with ClO$_x$ produce ClO$_3^-$, not ClO$_4^-$.

2.5.4.11 $\Delta^{17}$O in UV Experiments

**UV-Hypochlorite**

The reactions involved in the photodecomposition of OCl$^-$ are well-known and possible ClO$_4^-$ formation mechanisms have been proposed from them (Kang et al., 2006; Buxton et al., 1972a,b). These ClO$_4^-$ mechanisms have been compiled into one diagram (Figure 2.5.24) and are dependent upon the wavelength (253.7 nm and 313 nm, or 365 nm) at which initial photolysis of OCl$^-$ occurs (Figure 2.5.24; Rxn’s 1-3). Three pathways (Figure 2.5.24; [1] Rxn sequence: 1, 4-5, 25, 30, and 41-43, [2] Rxn sequence: 1, 4-6, 26, 30, and 41-43, [3] Rxn sequence: 1, 4-6, 20, 30, and 41-43) are possible at low wavelengths (253.7 and 313 nm) and depend on Cl atoms to oxidize OCl$^-$, while two pathways (Figure 2.5.24; [1] Rxn sequence: 3, 13, 17, 21, 30, and 41-43, [2] Rxn sequence: 3, 13, 20, 30, and 41-43) are predominant at a higher wavelength (365 nm) and depend on the ground state O(3P) atom to oxidize OCl$^-$.

The low $\Delta^{17}$O values (- 0.0 ‰ and + 0.5 ‰) of ClO$_4^-$ formed from UV oxidation of OCl$^-$ indicated no involvement of O$_3$ and thus, it was determined that pathways leading to ClO$_4^-$ did not form O$_3$ and therefore, could not be postulated on the basis of the number of O atoms coming from the O$_3$ molecule. However, because UV experiments were conducted at a wavelength of 350 nm, the pathways most likely involved in production of ClO$_4^-$ are believed to be the two
involving the oxidation of OCl⁻ by the ground state O(³P) atom (Figure 2.5.24; [1] Rxn sequence: 3, 13, 17, 21, 30, and 41-43, [2] Rxn sequence: 3, 13, 20, 30, and 41-43). Similar to the pathways for O₃ oxidation of OCl⁻, the formation of two key intermediate species was found necessary for ClO₄⁻ production in the UV experiments: ClO₂ and the ClO₃ radical (Figure 2.5.24). The only uncertainty associated with the two O(³P)-ClO₄⁻ pathways is as to which reactions are responsible for the formation of ClO₂: Rxn’s 17 and 21 or Rxn 20 (Figure 2.5.24). The reactions following ClO₂ formation, which included oxidation of ClO₂ into the ClO₃ radical (Rxn’s 30 and 41), the reaction between two ClO₃ radicals to produce Cl₂O₆ (Rxn 42), and the hydrolysis of the Cl₂O₆ species (Rxn 43) were common and deemed necessary for the production of ClO₄⁻ in all the UV mechanisms proposed.
Figure 2.5.24. UV oxidation of OCl⁻ (aq). Note the thick red lines indicate the pathways leading to the formation of Cl⁻, the thick aqua lines indicate the pathways leading to formation of ClO₃⁻, and the thick blue lines indicate the pathways leading to the formation of ClO₄⁻. The solid lines indicate reactions specific only to the anion produced, the dashed lines indicate shared reactions between pathways leading to either two or all three anions, and the black solid circles represent reaction numbers corresponding to reactions listed.
UV-Chlorite
Photodecomposition pathways of ClO$_2^-$ have also been previously proposed and are summarized in Figure 2.5.25 (Kang et al., 2006). Determination of the exact pathway(s) responsible for ClO$_4^-$ formation from UV oxidation of ClO$_2^-$ is not possible based on the number of O atoms from O$_3$ as the $\Delta^{17}$O values of ClO$_4^-$ produced were below zero (~ - 2%), indicating that UV pathways are mass-dependent processes and that O$_3$ was not a major source of O in ClO$_4^-$. The photolysis of ClO$_2^-$ was observed at a wavelength of 350 nm and thus the ClO$_4^-$ pathways proposed only include reactions occurring at longer wavelengths (365 nm) (Figure 2.5.25; Rxn’s 17 and 18). All preferred UV ClO$_4^-$ pathways lead to the formation of the ClO$_2$ molecule which undergoes the same final reaction sequence (Figure 2.5.25; Rxn’s 30, and 41 – 43) as the pathways for UV oxidation of OCl$^-$ (Figure 2.5.24) to produce ClO$_4^-$. 
Figure 2.5.25. UV oxidation of ClO$_2^-$ (aq). Note the thick red lines indicate the pathways leading to the formation of Cl$^-$, the thick aqua lines indicate the pathways leading to formation of ClO$_3^-$, and the thick blue lines indicate the pathways leading to the formation of ClO$_4^-$. The solid lines indicate reactions specific only to the anion produced, the dashed lines indicate shared reactions between pathways leading to either two or all three anions, and the black solid circles represent reaction numbers corresponding to reactions listed.
**UV-Chlorine Dioxide**

$\Delta^{17}O$ values of ClO$_4^-$ formed from the photodecomposition of ClO$_2$ (aq) (+ 0.9 ‰ and + 1.3 ‰) were higher than those of ClO$_4^-$ from the OCl$^-$ and ClO$_2^-$ experiments indicating either an unusual mass-dependent process or possibly that O$_3$ was generated by the UV reactions. These $\Delta^{17}O$ values provide further evidence indicating that ClO$_4^-$ produced from UV pathways is most likely due to mass dependent fractionation processes and thus cannot be formulated on the basis of O derived from O$_3$. Based on what we know regarding UV oxidation of OCl$^-$ and ClO$_2^-$, only one pathway was determined possible for the formation of ClO$_4^-$ from UV oxidation of ClO$_2$ (aq) (Figure 2.5.26; Rxn sequence: 30 and 41-43). This pathway requires the formation of the ClO$_3$ radical (rxn 41) and the Cl$_2$O$_6$ species (rxn 42).
Figure 2.5.26. UV oxidation of ClO$_2$ (aq). Note the thick red lines indicate the pathways leading to the formation of Cl$^-$, the thick aqua lines indicate the pathways leading to formation of ClO$_3^-$, and the thick blue lines indicate the pathways leading to the formation of ClO$_4^-$. The solid lines indicate reactions specific only to the anion produced, the dashed lines indicate shared reactions between pathways leading to either two or all three anions, and the black solid circles represent reaction numbers corresponding to reactions listed.
2.5.4.12 Implications of $\delta^{18}O$ and $\Delta^{17}O$

**Expected Relationship Between $\delta^{18}O$ and $\Delta^{17}O$ Values of ClO$_4^-$**
For all experiments (UV and O$_3$) in which O isotopes were measured, the $\delta^{18}O$ values of ClO$_4^-$ were much higher than the initial total reactant ClO$_x$ species, bulk water, and produced ClO$_3^-$ (the only other final ClO$_x$ species (Figures 2.5.5 – 2.5.12). With the exception of $\delta^{18}O$ values of ClO$_4^-$ from the oxidation of ClO$_2$ (aq), in the O$_3$ experiments, the $\Delta^{17}O$ and $\delta^{18}O$ values of ClO$_4^-$ increased as oxidation state of Cl in reactant ClO$_x$ species (ClO$_2^-$, OCl$^-$, and Cl$^-$, respectively) decreased, whereas in the UV experiments, as expected, no trend was observed between $\Delta^{17}O$ and $\delta^{18}O$ values (Figures 2.5.13 – 2.5.15). The increase in $\delta^{18}O$ values with increasing number of O incorporated from O$_3$ is expected. The $\delta^{18}O$ values for experiments involving O$_3$ should be proportional to the number of O atoms incorporated from O$_3$, although the relationship may not be linear given possible variation in the $\delta^{18}O$ values of other O sources and possible impacts of exchange and/or fractionation. Interpretations of the $\delta^{18}O$ values of ClO$_4^-$ formed from each individual reactant ClO$_x$ species for both oxidation methods (O$_3$ and UV) were attempted by evaluating the likelihood of these three factors. Where possible (O$_3$ experiments) we attempted to evaluate the possible impact of O from other sources by using the expected number of O atoms from O$_3$ and other sources and applying an isotope mass balance.

**O$_3$-Dry Chloride**
The O$_3$ oxidation of dry Cl$^-$ produced ClO$_4^-$ with the highest $\Delta^{17}O$ values (~33 ‰) which indicated most, if not all, O in ClO$_4^-$ is from O$_3$ depending on the final site preference value. If all the O were sourced from O$_3$, then $\delta^{18}O$ values of ClO$_4^-$ should have been near + 124 ‰, assuming no fractionation effects. The lower $\delta^{18}O$ values (+ 20.5 ‰) indicate that either some of the O came from a source with very negative values, that some O exchanged with an O source for which the equilibrium value is very negative, or that the O incorporated from O$_3$ was fractionated. Assuming that O$_3$ had a final $\Delta^{17}O$ site preference value of + 7 ‰, the maximum possible, it was determined (Equations 6 and 7) that a maximum of 10 % O from ClO$_4^-$ could have come from a source other than O$_3$.

Although caution was taken to remove H$_2$O in the reaction tubes used in the dry Cl$^-$ experiments, there is a possibility that some moisture remained in the tubes resulting in interactions between H$_2$O and ClO$_x$ species. If moisture was involved then four O sources for ClO$_4^-$ would have been available: O$_3$, H$_2$O, OH radical, and O$_2$. However, O$_2$ is not an oxidant in any of the ClO$_4^-$ production pathways from O$_3$ oxidation of Cl$^-$ (aq) and we assume that the O from the OH radical is from O$_3$, thus they were not considered further in our mixing evaluation.

Bulk water used in the experiment had a $\delta^{18}O$ value of - 6.9 ‰, the lowest of the known O sources, but residual H$_2$O on the salt would likely be much heavier. Therefore, even assuming
that the source of O was bulk water and using the maximum % of O that could come from water based on the maximum site preference value and the $\delta^{18}O$ mass balance (Equations 6 and 8) would result in a ClO$_4^-$ with a $\delta^{18}O$ value (+111 ‰) higher than the $\delta^{18}O$ value measured for ClO$_4^-$ (+20.5 ‰) (Figure 2.5.27). For mixing to have been responsible 79 % (3.16) of the O atoms in ClO$_4^-$ would have to be from H$_2$O and only 21 % (0.84) from O$_3$, therefore, contribution of O atoms from other sources cannot account for the $\delta^{18}O$ value of ClO$_4^-$ from O$_3$ oxidation of dry Cl$^-$. We do not have any information on possible O equilibrium exchange values and therefore, cannot evaluate their potential to account for the observed $\delta^{18}O$ ClO$_4^-$ values, but they would have to be substantially more negative than bulk H$_2$O. Given the above discussion, it appears that the most likely explanation for the relatively low observed $\delta^{18}O$ values, given the $\Delta^{17}O$ values, is the impact of mass dependent fractionation during ClO$_4^-$ formation.

$\%\ O\ from\ H_2O + \%\ O\ from\ O_3 = 1$  

(6)

($\%\ O\ from\ H_2O \times \Delta^{17}O\ of\ H_2O) + (\%\ O\ from\ O_3 \times \Delta^{17}O\ of\ O_3) = \Delta^{17}O\ of\ ClO_4^-$  

(7)

($\%\ O\ from\ H_2O \times \delta^{18}O\ of\ H_2O) + (\%\ O\ from\ O_3 \times \delta^{18}O\ of\ O_3) = \delta^{18}O\ of\ ClO_4^-$  

(8)
Figure 2.5.27. Stable O isotopic compositions ($\delta^{18}$O and $\Delta^{17}$O) of reactant species and final ClO$_4^-$ for O$_3$ oxidized ClO$_x$ solutions including expected $\delta^{18}$O and $\Delta^{17}$O of ClO$_4^-$ formed from the mixture of H$_2$O and O$_3$ (with complete site preference of the heavy O isotope in the terminal O atoms of O$_3$) (black circles) and expected $\delta^{18}$O and $\Delta^{17}$O of ClO$_4^-$ formed from the mixture of H$_2$O and O$_3$ (with no site preference of the heavy O isotope in the terminal O atoms of O$_3$) (green circles). The replicate experiments are represented by the different colors (blue and red). The species label next to the final ClO$_4^-$ symbols indicates the species oxidized corresponding to that ClO$_4^-$.

**O$_3$-Chloride (aq)**

Similar to dry Cl$^-$, $\Delta^{17}$O values of ClO$_4^-$ from the Cl$^-$ (aq) experiments (+29‰ and +32‰) also indicated that nearly all O atoms in ClO$_4^-$ were from O$_3$, depending on the final $\Delta^{17}$O site preference value of O$_3$. The ClO$_4^-$ $\delta^{18}$O values were however higher (~25.5 – 34.5‰) than those of ClO$_4^-$ from the dry Cl$^-$ experiments but they were still lower (~69 – 78‰) than what would be expected (~+124‰) assuming no fractionation effects. Assuming that O$_3$ had a final $\Delta^{17}$O site preference value of +37‰, the maximum possible, it was determined using a $\Delta^{17}$O
mass balance that between 13–21% of O in ClO$_4^-$ could come from H$_2$O and the other 79–87% from O$_3$ (Equations 6 and 7). It was determined through a $\delta^{18}$O mass balance that even assuming the maximum % of O that could come from H$_2$O (21%) would result in a ClO$_4^-$ with a $\delta^{18}$O value of +97‰, a value much greater than the ClO$_4^-$ $\delta^{18}$O values observed (+46‰ to +55‰) (Figure 2.5.27). Therefore it was concluded that mixing of O sources most likely does not account for the $\delta^{18}$O values of ClO$_4^-$ formed from O$_3$ oxidation of Cl$^-(aq)$, but that given the little that is known regarding O exchange between ClO$_x$ species, that mass-dependent fractionation effects are the most likely explanation for the $\delta^{18}$O values of ClO$_4^-$. 

**O$_3$-Hypochlorite**

The $\Delta^{17}$O values of ClO$_4^-$ from O$_3$ oxidation of OCl$^-$ (+16‰, +17‰, and +20‰) indicated that, assuming a $\Delta^{17}$O site preference value of +32‰ for O$_3$ based on the Cl$^-$ oxidation experiments, two to three O in ClO$_4^-$ come from O$_3$. The ClO$_4^-$ $\delta^{18}$O values observed (+1‰, +2‰, and +11‰) were much lower than those of O$_3$ (+124‰), but higher than those of H$_2$O (-6.9‰) and initial total O (-28‰), all of which are species considered as O sources available for ClO$_4^-$ formation. Because less than 5% of the initial total O was attributed to a species other than OCl$^-$, the $\delta^{18}$O values of initial total O were considered to be the same for OCl$^-$ (-28‰).

To evaluate whether mixing of O sources could account for the ClO$_4^-$ $\delta^{18}$O values observed, a mass balance on $\Delta^{17}$O values of OCl$^-$ (-0.07‰ to +0.4‰) (assuming the original O in the reactant OCl$^-$ species was transferred into final ClO$_4^-$), O$_3$ (assuming a maximum possible $\Delta^{17}$O site preference value of +37‰), and H$_2$O (0‰) was performed. Assuming that 25% of the O in ClO$_4^-$ came from OCl$^-$, the mass balance indicates that 19.5–30% of O came from H$_2$O, and 45–55.5% of O came from O$_3$ (Equations 9 and 10). Assuming the minimum % of O that could come from OCl$^-$ (25%) and the maximum % that could come from H$_2$O (30%), it was determined through a $\delta^{18}$O mass balance that ClO$_4^-$ should exhibit a $\delta^{18}$O value near +47‰, a value much higher than the ClO$_4^-$ $\delta^{18}$O values observed (+1‰, +2‰, and +11‰).

Given these results, it is unlikely that mixing of O$_3$ with other O sources (OCl$^-$ and H$_2$O) alone is responsible for the $\delta^{18}$O values of ClO$_4^-$ formed from O$_3$ oxidation of OCl$^-$. Therefore, based on $\Delta^{17}$O values indicating only partial contribution of O$_3$-O atoms into ClO$_4^-$ and the mixing calculations, we conclude that the other most likely explanation for the observed ClO$_4^-$ $\delta^{18}$O values is the impact of mass dependent fractionation during ClO$_4^-$ formation.

\[
\% \text{ O from } H_2O + \% \text{ O from } O_3 + \% \text{ O from OCl}^- = 1
\]  

(9)
\[
\% O \text{ from } H_2O \times \Delta^{17}O \text{ of } H_2O) + \% O \text{ from } O_3 \times \Delta^{17}O \text{ of } O_3 \times \Delta^{17}O) = \Delta^{17}O \text{ of } ClO_4^- \tag{10}
\]

\[
\% O \text{ from } H_2O \times \delta^{18}O \text{ of } H_2O) + \% O \text{ from } O_3 \times \delta^{18}O \text{ of } O_3 \times \delta^{18}O) = \delta^{18}O \text{ of } ClO_4^- \tag{11}
\]

**O₃-Chlorite**

The O₃ oxidation of ClO₂⁻ produced ClO₄⁻ with the lowest Δ¹⁷O values (+ 12 ‰ and + 13 ‰) which indicated that two or less O in ClO₄⁻ is from O₃, depending on the final site preference value of O₃. Assuming a Δ¹⁷O site preference value of + 37 ‰ for O₃, the maximum possible and the measured Δ¹⁷O of H₂O (0‰) and ClO₂⁻ (+ 0.0 ‰) (assuming that the original O in the reactant ClO₂⁻ species was transferred into the final ClO₄⁻ structure), a mass balance determined that 15 – 17 % of O came from H₂O, and 33–35 % of O came from O₃ (Equations 12 and 13).

Out of the three O sources available (ClO₂⁻, H₂O, and O₃), bulk water has the lowest δ¹⁸O value (- 6.9 ‰). Assuming the maximum % of O that could come from water (17 %), based on the Δ¹⁷O mass balance, and the assumed 50 % of O that came from ClO₂⁻, we would expect a ClO₄⁻ with a δ¹⁸O value near + 39 ‰, a value considerably greater than the measured ClO₄⁻ δ¹⁸O values (+ 2 ‰ and + 4 ‰). The ClO₄⁻ Δ¹⁷O values indicate that another O source other than O₃ is involved in the formation of ClO₄⁻, but given the mass balance results it appears that mixing alone cannot be responsible for the δ¹⁸O values observed. Given previous results for other oxidized species (Cl⁻ and OCl⁻) the most likely explanation is that mass dependent fractionation and mixing of O from the original ClO₂⁻ were both responsible for the δ¹⁸O values of ClO₄⁻ from O₃ oxidation of ClO₂⁻.

\[
% O \text{ from } H_2O + % O \text{ from } O_3 + % O \text{ from } ClO_2^- = 1 \tag{12}
\]

\[
(\% O \text{ from } H_2O \times \Delta^{17}O \text{ of } H_2O) + (\% O \text{ from } O_3 \times \Delta^{17}O \text{ of } O_3) + (\% O \text{ from } ClO_2^- \times \Delta^{17}O) = \Delta^{17}O \text{ of } ClO_4^- \tag{13}
\]

\[
(\% O \text{ from } H_2O \times \delta^{18}O \text{ of } H_2O) + (\% O \text{ from } O_3 \times \delta^{18}O \text{ of } O_3) + (\% O \text{ from } ClO_2^- \times \delta^{18}O) = \delta^{18}O \text{ of } ClO_4^- \tag{14}
\]
**O₃-Chlorine Dioxide**

The Δ¹⁷O values of ClO₄⁻ from O₃ oxidation of ClO₂ (aq) (+16 to +17 ‰) were similar to those of ClO₄⁻ from O₃ oxidation of OCl⁻ (+16, +17, and +20‰) but were somewhat higher than those of ClO₄⁻ from O₃ oxidation of ClO₂⁻ (+12 ‰ and +13 ‰). However, the δ¹⁸O values of ClO₄⁻ formed in the ClO₂ (aq) experiments (+23‰ to +28‰) were higher than the δ¹⁸O values of ClO₄⁻ from both O₃ oxidation of OCl⁻ and ClO₂⁻: (+1‰, +2‰, and +11‰) and (+2‰ and +4‰), respectively. It is not possible to evaluate the likelihood of O source mixing, fractionation, and/or O exchange in the ClO₂ (aq) experiments as ¹⁸O isotopes for the starting ClO₂ (aq) were not measured.

**UV-Hypochlorite**

Photodecomposition of OCl⁻ produced a ClO₄⁻ with near zero Δ¹⁷O values (-0.0‰ to +0.5‰) and the lowest δ¹⁸O values (-19‰ to -22‰) out of all the UV experiments. ClO₄⁻ pathways proposed for UV oxidation of OCl⁻ (Figure 2.5.24) indicate at least five O sources are available for ClO₄⁻ formation: initial OCl⁻, O(³P), OH, OCl, and H₂O. Initial OCl⁻ and H₂O had low δ¹⁸O values (-28‰ and -6.9‰, respectively), but the Δ¹⁷O and δ¹⁸O values of O(³P), OH, and OCl are not available, thus a proper evaluation of the percentage of O in ClO₄⁻ from the different O sources cannot be made.

**UV-Chlorite**

The photolysis of ClO₂⁻ led to the production of ClO₄⁻ with Δ¹⁷O values relatively close to zero (-1‰ to -2‰) but with high δ¹⁸O values (+42‰ to +46‰). Only three O sources were identified in the proposed ClO₄⁻ mechanisms (Figure 2.5.25) as available for ClO₄⁻ formation from UV exposure to ClO₂⁻: initial ClO₂⁻, the OCl radical, and H₂O. Because there is lack of information related to the δ¹⁸O values of the OCl radical, that might have been an intermediate product in the experiment, it is not possible to determine the likelihood of mixing between these three O sources. However, because the δ¹⁸O values of initial ClO₂⁻ (-2.5‰) and H₂O (-6.9‰) were both below zero it can be deduced that for the ClO₄⁻ formed to be a result of mixing of the O sources, the δ¹⁸O value of the OCl radical would have to be higher than the δ¹⁸O values of the ClO₄⁻ reagent or that there was substantial O isotope fractionation.

**UV-Chlorine Dioxide**

The photodecomposition of ClO₂ (aq) resulted in ClO₄⁻ with Δ¹⁷O values near zero (+0.9‰ and +1.3‰) and with δ¹⁸O values (+9‰ to +13‰) higher than those of the OCl⁻ experiments (-19‰ to -22‰) but lower than those of the ClO₂⁻ experiments (+42‰ to +46‰). Two initial O sources were available in the UV ClO₂ (aq) experiments: initial ClO₂ (aq) and H₂O. The δ¹⁸O values for initial ClO₂ are not available and thus a proper evaluation of mixing of the O sources cannot be made. One conclusion that can be drawn is that the δ¹⁸O values of initial ClO₂ (aq)
would have to be higher than the $\delta^{18}O$ values of H$_2$O and ClO$_4^-$ for mixing to be responsible for the ClO$_4^-$ $\delta^{18}O$ values.

2.5.4.13 Fractionation

There was not enough information in the UV experiments to form a thorough evaluation of the $\delta^{18}O$ values of ClO$_4^-$ formed from oxidation of all ClO$_x$ species (OCl$^-$, ClO$_2^-$, and ClO$_2$ (aq)), but given the mixing $\delta^{18}O$ results, it appears that the $\delta^{18}O$ values for ClO$_4^-$ in the O$_3$ experiments are in line with simple mass-dependent fractionation where ClO$_4^-$ gets lighter than would be expected given the maximum $\Delta^{17}O$ site preference value of O$_3$ (+ 37 ‰). It is possible that ClO$_4^-$ $\delta^{18}O$ values in O$_3$ experiments were lower due to the continuous input of O$_3$ into the reaction vessels. Fractionation would dictate that the lighter O isotope would react first and thus continuous input would purge out the already reacted O$_3$, allowing for a cycle of the lighter O isotope of the new O$_3$ to keep reacting.

Kinetic isotope fractionation would result in a large fractionation effect, depending on the pathway leading to ClO$_4^-$, the reaction rates of reactions involved, and the bonds of species being broken or formed in each reaction (Kendal et al., 2011). Not all reaction rates for reactions in the proposed ClO$_4^-$ formation mechanisms (Figure 2.5.16–2.5.23) are known and reaction sequences are speculative, thus it is not possible to determine if $\delta^{18}O$ values of ClO$_4^-$ resulted from kinetic fractionation and/or equilibrium isotope exchange reactions.

Implications of $\delta^{37}Cl$

With the exception of the O$_3$ dry Cl$^-$ experiments the $\delta^{37}Cl$ values of ClO$_4^-$ formed in both aqueous UV and O$_3$ experiments were higher than the $\delta^{37}Cl$ values of the source species (Cl$^-$, OCl$^-$, ClO$_2^-$, and ClO$_2$) (Figures 2.5.5–2.5.12). The $\delta^{37}Cl$ values of ClO$_4^-$ formed in the UV experiments were generally higher than the $\delta^{37}Cl$ values of ClO$_4^-$ produced in the O$_3$ experiments, but ClO$_4^-$ $\delta^{37}Cl$ values from the different photolyzed ClO$_x$ species were similar to one another (Figure 2.5.15). In the O$_3$ experiments the $\delta^{37}Cl$ values of ClO$_4^-$ appear to be roughly correlated with the oxidation state of the Cl in the reactant species, with the only exception being the $\delta^{37}Cl$ values of ClO$_4^-$ from the ClO$_2^-$ experiments.

The high $\delta^{37}Cl$ values of ClO$_4^-$ produced in the UV and O$_3$ experiments may have been influenced by equilibrium or kinetic fractionations between different aqueous ClO$_x$ species. Mixing calculations are not possible due to the lack of $^{37}Cl$ isotope data for all possible Cl sources in both UV and O$_3$ experiments, but it is hypothesized that because $\delta^{37}Cl$ values of ClO$_4^-$ produced in all aqueous O$_3$ and UV experiments were relatively high, that the $\delta^{37}Cl$ values of the Cl sources would need to be relatively high as well for mixing to have occurred while in the O$_3$
dry Cl\textsuperscript{−} experiment, the $\delta^{37}$Cl values of the Cl source would be expected to be below zero. Given that our source Cl was typically near zero, it is unlikely that it had an impact on our $\delta^{37}$Cl values.

Kinetic Cl isotope fractionation factors related to photodecomposition or O\textsubscript{3} oxidation of Cl\textsuperscript{−}, OCI\textsuperscript{−}, ClO\textsubscript{2}\textsuperscript{−}, and ClO\textsubscript{2} have not been experimentally determined. Schauble et al.\textsuperscript{49} determined that under equilibrium conditions at 298 K, $\delta^{37}$Cl values of coexisting ClO\textsubscript{x} species were possibly correlated with Cl oxidation state: Cl\textsuperscript{−1}, Cl\textsuperscript{0}, Cl\textsuperscript{+1}, Cl\textsuperscript{+2}, Cl\textsuperscript{+3}, Cl\textsuperscript{+4}, and Cl\textsuperscript{+7} can concentrate $^{37}$Cl approximately by +2.5‰ to +6‰, +7‰, +7‰ to +9‰, +10‰, +27‰, and +75‰, respectively. Any reversible reaction in the proposed ClO\textsubscript{4}\textsuperscript{−} mechanisms for either UV or O\textsubscript{3} processes (Figures 2.5.16–2.5.26) has the potential to have experienced equilibrium isotope exchange. Although it is not known if the proposed reversible reactions (Table 2.5.4: Rxns 1, 3–4; and UV Rxn’s 5 and 26) reached full equilibrium, even if only partial equilibrium occurred, the $\delta^{37}$Cl values of ClO\textsubscript{4}\textsuperscript{−} produced in the aqueous UV (+17‰ to +23‰) and O\textsubscript{3} (+5‰ to +17‰) experiments might be reasonable given the Cl isotope fractionations calculated by Schauble et al, 2003.

It is possible that some of the proposed reactions may have created intermediate species that were then consumed by multiple branching reactions with different isotope effects, continuously getting heavier and thus forming heavier ClO\textsubscript{4}\textsuperscript{−}. One example of this is reaction 4 (Table 2.5.4) from the O\textsubscript{3} proposed mechanisms, a reaction for which no forward or reverse reaction rates are known. If ClO\textsubscript{2} was converted to ClO\textsubscript{3} with relatively little kinetic isotope fractionation while also being consumed by another reaction with a larger kinetic fractionation, the $\delta^{37}$Cl of ClO\textsubscript{3} could increase rapidly and thus produce ClO\textsubscript{4}\textsuperscript{−} with high $\delta^{37}$Cl values. This is purely hypothesized but illustrates that high $\delta^{37}$Cl ClO\textsubscript{4}\textsuperscript{−} could be produced in a complex reaction network.

Schauble et al., 2003 also suggested that under equilibrium some molecules could have different affinities for the heavier isotopes, thus it is also possible that molecules that break apart to form two or more species could result in one product species being heavier than the other, depending on the location of the heavy isotope. This would be more applicable to the UV experiments as all ClO\textsubscript{4}\textsuperscript{−} pathways proposed go through the same final reaction sequence (Figures 2.5.24–2.5.26; rxn’s 41–43) that ends up in the Cl\textsubscript{2}O\textsubscript{6} species being hydrolyzed to produce ClO\textsubscript{4}\textsuperscript{−} and ClO\textsubscript{3}\textsuperscript{−}. In this reaction (Rxn 43) the $^{37}$Cl isotope may be located in the Cl\textsubscript{2}O\textsubscript{6} species in such a way that when hydrolyzed, always ends up in ClO\textsubscript{4}\textsuperscript{−}. Once formed ClO\textsubscript{4}\textsuperscript{−} is not known to further react thus we do not know if this phenomenon is possible outside of equilibrium.

Given the high $\delta^{37}$Cl values of ClO\textsubscript{4}\textsuperscript{−} from the aqueous UV and O\textsubscript{3} experiments and the low $\delta^{37}$Cl values of ClO\textsubscript{4}\textsuperscript{−} from the O\textsubscript{3} dry Cl\textsuperscript{−} experiments, it appears that heterogeneous surfaces can undergo different isotope fractionation processes than do homogenous surfaces. The low $\delta^{37}$Cl
value of ClO$_4^-$ produced from Cl$^-$ in the dry UV experiments implies kinetic fractionations were more important than equilibrium effects in the dry system.

**Relation to Natural ClO$_4^-$**

All ClO$_4^-$ generated in the UV and O$_3$ aqueous experiments was as heavy as or heavier in $\delta^{37}$Cl than natural ClO$_4^-$ (Figure 2.5.28). Our ClO$_4^-$ $\delta^{37}$Cl isotope data further suggest that aqueous production mechanisms produce ClO$_4^-$ that is enriched in $^{37}$Cl whereas dry production mechanisms produce ClO$_4^-$ depleted in $^{37}$Cl. Given this information, if aqueous reactions were solely responsible for ClO$_4^-$ production in the atmosphere and for natural ClO$_4^-$, then it would mean that precursor species, prior to ClO$_4^-$ formation, would need to have considerably lower $\delta^{37}$Cl values than Atacama ClO$_4^-$, which has the lowest reported $\delta^{37}$Cl values of any substance on Earth (Böhlke et al., 2005). Although it is possible that an unknown precursor species exists on Earth that is much lighter in $\delta^{37}$Cl than Atacama ClO$_4^-$, it is difficult to conceive an atmospheric production process that is purely dependent upon aqueous reactions. Given the lower $\delta^{37}$Cl values of ClO$_4^-$ from the O$_3$ dry Cl$^-$ experiments and those of natural ClO$_4^-$, it is more likely that natural ClO$_4^-$ is a result of dry (heterogeneous) atmospheric reactions (Figure 2.5.28). Dry production mechanisms could potentially start with a precursor species with high $\delta^{37}$Cl values, as high as those reported for species found on Earth, and would end up producing a ClO$_4^-$ with lower $\delta^{37}$Cl values, a finding that is more consistent with the reported $\delta^{37}$Cl values of natural ClO$_4^-$. 
Figure 2.5.28. Summary of isotope data for ClO₄⁻ produced in the O₃ (square symbols) and UV (circle symbols) generation experiments. Species labels next to the ClO₄⁻ symbols represent the precursor species from which ClO₄⁻ was produced. Generation data is displayed with previously published isotope data for ClO₄⁻ produced electrochemically (Böhlke et al., 2005) or found in natural samples from: (1) SHP and MRGB of western Texas and central New Mexico (Jackson et. al., 2010; Section 2.2 this report; Böhlke et al., 2005), respectively, (2) Atacama Desert, Chile (Böhlke et al., 2005, Bao and Gu, 2004) and (3) Death Valley (Jackson et. al., 2010; Section 2.1 and 2.3 this report).
All the $\delta^{18}O$ and $\Delta^{17}O$ values of ClO$_4^-$ produced in the O$_3$ experiments fell along a diagonal, positive line indicating different mixing reactions between O$_3$ and other O sources along with possible fractionation and/or isotope exchange (Figure 2.5.28). Our O$_3$ experiment $\delta^{18}O$ and $\Delta^{17}O$ isotope data is consistent with the trend observed between $\delta^{18}O$ and $\Delta^{17}O$ values of ClO$_4^-$ found in Death Valley and the Atacama caliche, indicating that ClO$_4^-$ in these regions consistent with O$_3$ production mechanisms (Figure 2.5.28). ClO$_4^-$ from the UV experiments did not have positive $\Delta^{17}O$ values but the $\delta^{18}O$ values largely bracketed the $\delta^{18}O$ values observed for MRGB/SHP ClO$_4^-$, indicating that indigenous ClO$_4^-$ in this region could be consistent with UV production mechanisms (Figure 2.5.28). Coincidentally, natural ClO$_4^-$ that is consistent with O$_3$ production mechanisms (Atacama and Death Valley) had negative $\delta^{37}Cl$ values while natural ClO$_4^-$ that is consistent with UV mechanisms (MRGB/SHP) had positive $\delta^{37}Cl$ values. Because concentrations and isotopic compositions of precursor species likely vary in the Earth’s atmosphere, we cannot account for which precursor species was actually responsible for each type of natural ClO$_4^-$ found on Earth.

**Uncertainty Related to $\Delta^{17}O$ and $\delta^{18}O$ Measurements**

Oxygen isotopes ratios in our study were analyzed using two IRMS methods: (1) the CO continuous-flow isotope-ratio mass spectrometry (CO-CFIRMS) method which is used to measure $\delta^{18}O$ and (2) the O$_2$ dual-inlet isotope-ratio mass spectrometry (O2-DIIRMS) method which is used to measure $\delta^{17}O$ and $\delta^{18}O$ (Bohlke et al., 2016). The average difference between $\delta^{18}O$ values from each method was 1.55 ± 1.29 ‰. Only the $\delta^{18}O$ values obtained from the O2-DIIRMS method were reported and used for $\Delta^{17}O$ calculations in this study as $\delta^{18}O$ values from the CO-CFIRMS method were not available for all species.

$\Delta^{17}O$ values are also considered an effect of different equations and different coefficients/exponents (λ) (Table 2.5.5). Many definitions of $\Delta^{17}O$ have been formulated, with the coefficient (λ) ranging from 0.50 – 0.53 depending on the type of conditions and processes being observed, and can result in different $\Delta^{17}O$ values (Table 2.5.5) (Miller et al., 2002; Cliff et al., 1997; Farquhar et al., 1999; Luz et al., 2005). In our study we adopted the definition of Miller et al., 2002 and assumed a λ value of 0.525. Because $\Delta^{17}O$ variations in values of ClO$_4^-$ from the UV experiments are small, we must consider the variability in $\Delta^{17}O$ values of ClO$_4^-$ had we used other definitions. The $\Delta^{17}O$ values of ClO$_4^-$ calculated using different definitions of $\Delta^{17}O$ and λ range of 0.50 – 0.53 are reported in Table 2.5.5 and indicate that the photodecomposition of OCl$_{-}$ might be the only process associated with solely mass-dependent fractionation, as it was the only experiment for which replicates had values near zero (greater than -0.5 ‰ but less than 0.5‰). Regardless of the definition, it appears that $\Delta^{17}O$ values of ClO$_4^-$ from the UV ClO$_2^-$ and ClO$_2$ (aq) experiments are truly negative and positive, respectively, indicating some mass-independent fractionation (Table 2.5.5).
Table 2.5.5. Calculated $\Delta^{17}$O values of ClO$_4^-$ produced in the UV experiments using the coefficient ($\lambda$) range of 0.50 – 0.53 in the various definitions of $\Delta^{17}$O.

<table>
<thead>
<tr>
<th>Definition/Equation</th>
<th>Reference</th>
<th>Possible Range in $\Delta^{17}$O Values of ClO$_4^-$ in UV Experiments (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OCl$^-$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep 1</td>
</tr>
<tr>
<td>$\Delta^{17}$O = $k = \frac{(1 + \delta^{17}O)}{(1 + \delta^{18}O)^2} - 1$</td>
<td>Miller, 2002</td>
<td>(-0.52) - (+0.07)</td>
</tr>
<tr>
<td>$\Delta^{17}$O = $\delta^{17}O - (\lambda \times \delta^{18}O)$</td>
<td>Cliff and Thiemens, 1997 Thiemens, 1999</td>
<td>(-0.56) - (+0.02)</td>
</tr>
<tr>
<td>$\Delta^{17}$O = (1 + $\delta^{17}O$) - (1 + $\delta^{18}O$)</td>
<td>Farquhar et al., 1999</td>
<td>(-0.51) - (+0.07)</td>
</tr>
<tr>
<td>$^{17}A = \ln(k + 1) = \ln(1 + \delta^{17}O) - \lambda \times \ln(1 + \delta^{18}O)$</td>
<td>Luz and Barkan, 2005</td>
<td>(-0.52) - (+0.07)</td>
</tr>
</tbody>
</table>

* Traditional definition which indicates deviation from a point on reference mass-dependent fractionation (MDF) line (Farquhar et al., 1999; Miller, 2002; Assonov and Breninkmeijer, 2005)

* This definition characterizes a specific mass-dependent fractionation (MDF) line, where $^{17}A$ is the ordinate intercept (Miller, 2002; Luz and Barkan, 2005; Assonov and Breninkmeijer, 2005)
2.5.5 Conclusions

This study presents proposed reaction mechanisms for the formation of ClO₄⁻, with distinct isotopic compositions (δ³⁷Cl, δ¹⁸O, and Δ¹⁷O), via O₃ oxidation and UV photolysis. While no new UV production pathways were introduced, we were able to partially constrain O₃ formation pathways based on the number of O atoms in ClO₄⁻ coming from O₃, as indicated by Δ¹⁷O values. The mechanisms proposed are ambiguous and there is still much information missing related to the intermediate species as well as the reaction rates for most of the reactions proposed.

Our findings suggest that ClO₄⁻ formed from oxidation (UV and O₃) of various ClOₓ species is a result of mixing between ClO₄⁻ formed from branching pathways along with some fractionation, with UV ClO₄⁻ isotope data suggesting mass-dependent fractionation and O₃ ClO₄⁻ isotope data being more consistent with mass-independent variation. There is also evidence to suggest that oxidation of ClOₓ species on a heterogeneous (dry) surface has different fractionation effects than oxidation of fully aqueous ClOₓ species.

From the Δ¹⁷O values of ClO₄⁻ formed in the O₃ experiments we were able to show a large O₃ position effect, indicating that the heavy isotope is preferentially located in the terminal O atoms in O₃ and that the terminal O atoms were preferentially incorporated into ClOₓ compounds. Δ¹⁷O values of ClO₄⁻ were approximately related to the number of O atoms added from O₃ and indicated a stepwise reduction in the contribution of O atoms from O₃ into ClO₄⁻ based on increasing oxidation state of Cl in precursor species. In addition, the Δ¹⁷O data for the O₃ experiments indicated large quantities of ClO₃⁻ were produced by a process that was not involved in production of ClO₄⁻, meaning that ClO₃⁻ was not the major precursor for ClO₄⁻ in the O₃ experiments. The δ³⁷Cl of ClO₄⁻ was generally higher than precursor δ³⁷Cl values in all experiments and was possibly a result of partial equilibrium reactions.

Although our experiments may not reflect natural conditions, they were successful in producing Cl and O isotopic variations in ClO₄⁻ that incorporate much of the reported variation in nature. The low δ³⁷Cl in Atacama ClO₄⁻ was not reproduced, but all other variations were. All other isotopic data suggests that ClO₄⁻ indigenous to Atacama and Death Valley and ClO₄⁻ from MRGB/SHP are consistent with an O₃ and a UV related ClO₄⁻ mechanism, respectively. Unfortunately we cannot determine which precursor species were involved in the formation of ClO₄⁻ in each of these regions. Our experiments showed that final ClO₄⁻ isotopic composition is dependent on the precursor species oxidized and given the global variations in precursor species in this planetary system, we cannot say for sure if ClO₄⁻ formed through mechanisms in our atmosphere would be the same. The reaction rates and intermediate species proposed to be involved in ClO₄⁻ formation require further study mixing, isotope exchange, and/or fractionation may be important during the formation process. More generation experiments involving
oxidation of other precursor species other than Cl\(^-\), on a heterogeneous surface or gas phase
would provide additional information to resolve ambiguities such as \(\delta^{37}\text{Cl}\) of Atacama Cl\(_2\text{O}_4^-\).
2.6 Evaluation of Bacterial Production of Perchlorate

2.6.1 Background

One potential production mechanism for natural ClO$_4^-$ that has not been previously evaluated is microbiological generation. Nitrifying bacteria generate nitrate through the oxidation of NH$_4$ (e.g., *Nitrosomonas* spp.) and NO$_2^-$ (e.g., *Nitrobacter* spp.) (Paerl, 1997). During this well-studied process, NH$_4$ serves as a microbial electron donor and oxygen as an electron acceptor (Capone, 1997). There are also anaerobic oxidation processes by which NO$_3^-$ is biologically generated from reduced nitrogen species. Moreover, a class of widely occurring haloperoxidase enzymes produced by specific fungi, algae, and bacteria are known to oxidize Cl$^-$ to hypochlorous acid (HClO) (Griffin, 1991; Vilter, 1995). This could represent an initial biological step in the production of natural ClO$_4^-$ in some environments (possibly followed by additional photochemical or biological reactions). We should also note that the oxygen isotope ratios reported for ClO$_4^-$ in West Texas are similar to those of biologically generated NO$_3^-$, which incorporates O from H$_2$O and O$_2$ during microbial oxidation of NH$_4$ (Hollocher et al., 1983; Kumar et al., 1983; Amberger and Schmidt, 1987; Böhlke et al., 2005). Thus, isotopic evidence supports the possibility of biologically generated ClO$_4^-$. During this task, we evaluated whether microbial generation of ClO$_4^-$ (as well as HClO, ClO$_2^-$, and ClO$_3^-$) is possible in an aerobic environment.

2.6.2 Initial Studies using Haloperoxidase Enzymes and Natural Sunlight

Experiments were conducted to evaluate whether specific haloperoxidase enzymes could produce ClO$_4^-$ during the oxidation of chloride (Cl$^-$), both in the presence and absence of ultraviolet (UV) light from natural sunlight. Haloperoxidases combine hydrogen peroxide (H$_2$O$_2$) with halides (e.g., Cl$^-$, I$^-$, Br$^-$) to form corresponding hypohalous acids (e.g., HClO from Cl$^-$) (Griffin, 1991; Vilter, 1995). Various microbial and plant species utilize this reaction as a protection mechanism from microbial pathogens (i.e., via acid formation) and as a means to chemically decompose complex polymers (Butler and Carter-Franklin, 2004). Initial experiments were conducted to determine if specific haloperoxidase enzymes could produce ClO$_4^-$ during the oxidation of Cl$^-$, both in the presence and absence of UV light. Follow-on studies were conducted to better refine conditions of ClO$_4^-$ generation. To our knowledge the potential for these enzymes to produce ClO$_4^-$, either as a minor side reaction, or through the subsequent interaction of the biologically-generated HClO$^-$ with UV light, has never been evaluated.
2.6.2.1 Methods

**Enzymes and buffer**

Two different haloperoxidase enzymes were initially tested. The first enzyme was a vanadium-based chloroperoxidase (VCP) derived from *Curvularia inaequalis*. A small aliquot of dried (~3 mg) VCP was provided to CB&I as gift by Tatyana Polenova at the University of Delaware. The entirety of this enzyme was suspended in 30 µl of assay buffer (see below). Only enough enzyme was available for a single experiment. The units of activity/ml for this preparation was not available. The second enzyme used was a iron-based chloroperoxidase derived from the filamentous fungus *Caldariomyces fumago* (FCP). This well-studied haloperoxidase was obtained from Sigma-Aldrich (St. Louis, MO). For initial experiments, this enzyme was suspended in assay buffer to an activity level of 7,000 units/ml. The assay buffer was prepared by first running distilled water through a column bed filled with Purolite A530E ion exchange resin to remove traces of ClO<sub>4</sub><sup>-</sup> from the laboratory water. Potassium phosphate (100 mM) and potassium chloride (50 mM) were then added to the water and the pH was adjusted to 5.0 with HCl.

**Experimental design**

A standard enzyme reaction consisted of 7,000 units of enzyme/ml assay buffer with 20 mM H<sub>2</sub>O<sub>2</sub>. The reactions were performed in 3 ml volumes ~5 ml quartz tubes (which do not block UV light) sealed with screw caps with a Teflon liner. In general, the enzyme was added as the final step. Deviations from this standard setup are noted in the results as appropriate. A number of controls were utilized to define the baseline level of ClO<sub>4</sub><sup>-</sup> in the test samples. The individual components (buffer, H<sub>2</sub>O<sub>2</sub>, and diluted enzyme) were analyzed separately. Furthermore, mixtures of the components (buffer + enzyme and buffer + H<sub>2</sub>O<sub>2</sub>) were also prepared and analyzed. Once all the additions were made, some samples were placed in the dark and incubated at 37°C, and others were incubated outdoors in direct sunlight for UV exposure. The temperature of the UV-exposed samples varied with the daily conditions. During the four initial experiments conducted, the influence of enzyme type, enzyme concentration, UV exposure, and H<sub>2</sub>O<sub>2</sub> concentration on the enzymatic production of ClO<sub>4</sub><sup>-</sup> were assessed. Results are detailed below.

**Analysis**

At the conclusion of each experiment, samples were analyzed for ClO<sub>4</sub><sup>-</sup> in the laboratory of Andrew Jackson at Texas Tech University using IC/MS/MS as described previously in this report. The detection limit for ClO<sub>4</sub><sup>-</sup> by this technique is ~ 0.05 µg/L.

2.6.2.2 Results and Discussion

All of the components that comprise the complete reaction mixture were analyzed for the presence of ClO<sub>4</sub><sup>-</sup> as separate entities (buffer, H<sub>2</sub>O<sub>2</sub>, and the enzyme mix diluted in water). The H<sub>2</sub>O<sub>2</sub> had the highest levels of ClO<sub>4</sub><sup>-</sup> (1.88 µg/L), while the diluted enzyme and buffer had much
lower concentrations (0.33 and 0.08 µg/L respectively). The contribution of ClO$_4^-$ from the H$_2$O$_2$ in the reaction mixture is ~1000x less than the reported concentration due to dilution (i.e., only 0.002 µg/L). Based on the initial results, the background ClO$_4^-$ concentration in the typical reaction mixture should equal ~0.4 µg/L or less.

In the initial study with vanadium chloroperoxidase (VCP), production of ClO$_4^-$ was not apparent when samples were incubated in the dark, but there was an increase in ClO$_4^-$ in those incubated for ~8 h in sunlight (Table 2.6.1). The mean ClO$_4^-$ concentration in the samples exposed to UV light (Full rxn – light in Table 2.6.1) was 0.86 µg/L whereas that in the samples remaining in the dark was 0.32 µg/L, which is similar to the control samples run for the study. It should be noted, however, that there was appreciable variability in the UV exposed samples, and the results were not statistically different from other samples. No additional enzyme was available to repeat this study.

In the subsequent experiments, Sigma-Aldrich fungal iron chloroperoxidase (FCP) enzyme was used. In the initial study, production of ClO$_4^-$ was evaluated in the presence and absence of UV light (Table 2.6.2). These data again showed significant variability among treatments, with mean ClO$_4^-$ concentrations ranging from 0.3 to 0.8 µg/L. The enzyme reaction performed in sunlight yielded higher ClO$_4^-$ than that performed in the dark (0.61 ± 0.09 µg/L vs 0.27 ± 0.08 µg/L, respectively), but both values were lower than that with buffer and H$_2$O$_2$ alone (i.e., no enzyme) at 0.76 ± 0.46 µg/L. In a subsequent study, the influence of H$_2$O$_2$ concentration on ClO$_4^-$ production was evaluated. All reactions were exposed to sunlight for ~8 h. As shown in Table 2.6.3, the ClO$_4^-$ concentrations ranging from ~1 to 4 µg/L in this study, which is appreciably higher than in the two previous studies. However, the samples again showed significant variability between replicates. A control sample without enzyme or not exposed to sunlight was not tested in this case.
Table 2.6.1. Evaluation of perchlorate production from H₂O₂ and Cl⁻ with vanadium chloroperoxidase (VCP).

<table>
<thead>
<tr>
<th>Sample</th>
<th>PC [ppb]</th>
<th>Range [ppb]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer + enzyme - dark</td>
<td>0.31</td>
<td>0.19</td>
<td>2</td>
</tr>
<tr>
<td>Buffer + enzyme - light</td>
<td>0.30</td>
<td>0.18</td>
<td>2</td>
</tr>
<tr>
<td>Buffer + H₂O₂ - dark</td>
<td>0.35</td>
<td>0.25</td>
<td>2</td>
</tr>
<tr>
<td>Buffer + H₂O₂ - light</td>
<td>0.34</td>
<td>0.23</td>
<td>2</td>
</tr>
<tr>
<td>Full rxn - dark</td>
<td>0.32</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Full rxn - light</td>
<td>0.86</td>
<td>0.6</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2.6.2. Evaluation of the influence of UV on perchlorate production by fungal iron chloroperoxidase.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ClO₄⁻ [µg/L]</th>
<th>Range [µg/L]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer + Enzyme</td>
<td>0.42</td>
<td>0.26</td>
<td>3</td>
</tr>
<tr>
<td>Buffer + H₂O₂</td>
<td>0.76</td>
<td>0.46</td>
<td>3</td>
</tr>
<tr>
<td>Full rxn - dark</td>
<td>0.27</td>
<td>0.08</td>
<td>3</td>
</tr>
<tr>
<td>Full rxn - light</td>
<td>0.61</td>
<td>0.09</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2.6.3. Evaluation of the influence of H₂O₂ concentration on perchlorate production by fungal iron chloroperoxidase.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ClO₄⁻ [µg/L]</th>
<th>Range [µg/L]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mM H₂O₂</td>
<td>1.02</td>
<td>0.41</td>
<td>2</td>
</tr>
<tr>
<td>2.5 mM H₂O₂</td>
<td>2.2</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td>12.5 mM H₂O₂</td>
<td>3.96</td>
<td>2.9</td>
<td>2</td>
</tr>
<tr>
<td>62.5 mM H₂O₂</td>
<td>2.85</td>
<td>3.3</td>
<td>2</td>
</tr>
</tbody>
</table>

All samples were exposed to sunlight and contained 7,000 units of enzyme.
2.6.3 Controlled Studies using Haloperoxidase Enzymes, Produced UV and Sunlight

In previous studies, two haloperoxidase enzymes were tested for their ability to produce ClO$_4^-$ in the presence/absence of sunlight and H$_2$O$_2$. The data showed high variability, but provided some indication that ClO$_4^-$ production was possible in samples incubated in sunlight with chloroperoxidase enzyme and H$_2$O$_2$. During these studies, the key sources of variability were hypothesized to be as follows: (1) incubation outdoors in sunlight as a source of UV without temperature control and, (2) addition of high concentrations of hydrogen peroxide in batch, as excess may cause destruction of the enzymatically-generated HClO (or ClO$^-$), which can subsequently be photochemically converted to ClO$_4^-$ by UV (see Chapter 2.1 reactions with hypochlorite + UV). Additional studies were subsequently conducted under better controlled conditions in order to reduce variability. In particular, a Rayonet photochemical reactor was utilized in the laboratory to better control UV exposure time, UV wavelength, and reaction temperature. In addition, H$_2$O$_2$ was added to the samples continuously at a low concentration using a laboratory syringe pump in order to minimize excess.

An assay was conducted with monochlorodimedone to evaluate the activity of the various chloroperoxidase enzymes. In the presence of hydrogen peroxide, chloroperoxidase enzymes have been observed to convert monochlorodimedone to dichlorodimedone, which can then be monitored via spectroscopic analysis (Hager et al., 1966).

2.6.3.1 Materials and Methods: Perchlorate Formation Assays

**Enzymes**

The following enzymes were used in these studies: Chloroperoxidase from *Caldariomyces fumago* (10,717 U/mL) was obtained from Sigma-Aldrich (St. Louis, MO) and from Bio-Research Products, Inc (21,972 U/mL; North Liberty, IA); and soybean peroxidase (1,356 U/mg) was obtained from Bio-Research Products, Inc. (North Liberty, IA).

**Buffers**

Phosphate buffer was made using water purified via a Barnstead UV filtration system (Barnstead, Dubuque, IA), which was then further treated to remove any trace ClO$_4^-$ by passing it through a column packed with Purolite A530E resin (Purolite, Bala Cynwyd, PA). The original buffer consisted of 100 mM potassium phosphate, dibasic (Fisher, Fair Lawn, NJ) plus 50 mM potassium chloride (Sigma-Aldrich). The phosphate buffer was later modified, with the modified buffer consisted of 10 mM potassium phosphate, dibasic, and 50 mM potassium chloride. The buffer pH was adjusted to pH 3.0, 5.0, or 7.0 using pure phosphoric acid (Fisher).

**Experimental Design: UV Reactor.** Triplicate chloroperoxidase enzyme assays were performed simultaneously by placing three quartz test tubes in a RAYONET photochemical chamber
reactor (Southern New England Ultra Violet Company, Branford, CT) equipped with two 300 nm UV bulbs. Tubes were placed approximately 45 mm away from the UV bulbs. Each tube contained an aliquot of peroxidase enzyme plus 2 mL phosphate buffer. Two lengths of Teflon® tubing were placed into each tube until they reached the bottom of the tube. One length of Teflon® tubing was attached to a peristaltic pump and was used to slowly bubble air through the buffer. The other length of Teflon® tubing was attached to a 20 mL plastic syringe containing buffer plus H₂O₂, which was metered into the test tubes at a rate of 0.083 mL/h (1 mL every 12 h). Experiments were conducted for 24 hours each. Samples were stored at 4°C until analysis.

Experimental Treatments
Experiments were performed in both 100 mM and 10 mM phosphate buffer at pH 3, pH 5, and pH 7. Controls consisted of experiments performed in the absence of H₂O₂, in the absence of enzyme, and in the absence of light (i.e., in the dark).
Sample Preparation. Upon initial sample analysis (see below), it was determined that the high levels of phosphate in the original buffer (containing 100 mM potassium phosphate) caused some interference with ClO₄⁻ analysis. A procedure was thus performed to reduce the amount of phosphate in the samples. First, the pH of the sample was raised to greater than pH 10 using a concentrated (10 N) sodium hydroxide solution. Twenty microliters of 5 M calcium chloride was then added per mL of sample. This caused the immediate precipitation of solid calcium phosphate, which was then removed from the samples by filtration through a 0.2 micron filter.

Experimental Design: Natural Sunlight
100 mM phosphate buffer was used in these studies as described above, except that buffer pH was adjusted to 2.75. A benchtop rotary shaker was placed outside in direct sunlight for UV exposure. A plastic tub containing ice packs was placed on the shaker. Eight 55 mm diameter quartz petri dishes (Technical Glass Products, Painesville, OH) were placed on top of the ice packs. Each dish contained 5 mL 100 mM phosphate buffer, hydrogen peroxide (final concentration 0.5 - 2 mM), and chloroperoxidase enzyme (final concentration 0.2 µg/mL). A quartz glass lid was placed on each dish, and four of the dishes were covered with aluminum foil to serve as no sunlight (i.e., dark) controls. The shaker was operated at 50 rpm for a period of four hours. Thermometers were used to measure both the temperature on the ice packs and the ambient outside temperature. Samples were removed at 2 h and 4 h and analyzed for perchlorate as previously described.

2.6.3.2 Materials and Methods: Monochlorodimedone (MCD) Assays

Buffers and Solutions
100 mM phosphate buffer was as described above for the sunlight studies, with the buffer pH was adjusted to 2.75. MCD solution was made by adding 3.62 mg of MCD (Bio-Research) per
mL to a 95% ethanol solution (e.g. 95% ethanol, 5% water). Peroxide solution consisted of 0.06 M hydrogen peroxide (Fisher) in water.

**Experimental Procedure**
A quartz cuvette containing 1 mL of phosphate buffer (100 or 10 mM) containing MCD (final concentration 0.0181 mg/mL) and H₂O₂ (final concentration 0.5 - 2 mM) was placed in the chamber of a Thermo Spectronic Genesys 2 Spectrophotometer (Thermo Scientific, Waltham, MA). The spectrophotometer was set to measure absorbance at 278 nm (A₂₇₈) every 5 sec. Chloroperoxidase enzyme (Bio Research; final concentration 1 µg/mL) was added immediately after absorbance measurements were initiated. A negative control experiment was performed in which enzyme was omitted (i.e., no enzyme control).

2.6.3.3 Results and Discussion

**UV Light Source Experiments**
Results of experiments performed using artificially generated UV light are presented in Table 2.6.4. Perchlorate levels in the experimental treatments were similar to those detected in the buffer alone, indicating that no significant ClO₄⁻ formation occurred. The background ClO₄⁻ concentration observed in the buffer is most likely a contaminant present in the phosphoric acid used to adjust the pH of the buffer.

**MCD Assays**
Results of MCD assays are presented in Figure 2.6.1. A rapid decrease in absorbance was observed when 2 mM peroxide and 100 mM phosphate buffer was used, indicating a reduction in monochlorodimedone concentrations as it is converted to dichlorodimedone (Hager et al., 1966). Significant decreases in A₂₇₈ were also observed when 2 mM peroxide and 0.01 M phosphate buffer (i.e., 1/10 strength phosphate buffer) and 1 mM phosphate buffer and 0.01 M phosphate buffer (i.e., 1/10 strength phosphate buffer and ½ strength peroxide) were used. MCD disappearance was significantly diminished when peroxide concentrations of 50 mM or less were used.

**Natural Sunlight Experiments**
Results of experiments performed using natural sunlight are presented in Table 2.6.5. Perchlorate levels in the experimental treatments (i.e., those exposed to natural sunlight) were similar to those detected in the controls, which were not exposed to light, indicating that no significant ClO₄⁻ formation was detected.

The results of the MCD assays indicated that the peroxidase enzymes being used in these studies were active under the study conditions used (i.e., at the testing buffer concentrations and pH),
and that their activity was rapid, with optimal MCD degradation occurring within the first 10 minutes of incubation. However, despite this, no significant ClO₄⁻ was formed when assays were exposed either to artificial UV light or to natural sunlight. The reason for this failure to produce ClO₄⁻ is unclear, but possible reasons include the inability of the peroxidase enzymes tested to produce significant hypochlorous acid, or rapid breakdown of that acid under test conditions before UV-mediated conversion to ClO₄⁻. The chloroperoxidase experiments were concluded at this point. It is possible that low levels of ClO₄⁻ are generated via this mechanism in nature, but unlikely that this is a significant source of natural ClO₄⁻ in the environment.

### Table 2.6.4. Perchlorate levels (µg/L) in UV light experiments.

<table>
<thead>
<tr>
<th>Buffer Used</th>
<th>pH</th>
<th>ClO₄⁻ in Buffer</th>
<th>Control Treatments</th>
<th>Experimental Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mM PO₄ buffer</td>
<td>5</td>
<td>0.18 ± 0.16*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>100 mM PO₄ buffer</td>
<td>7</td>
<td>0.48 ± 0.59*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10 mM PO₄ buffer</td>
<td>3</td>
<td>3.44 ± 0.03*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10 mM PO₄ buffer</td>
<td>5</td>
<td>0.15 ± 0.15*</td>
<td>0.28 ± 0.01</td>
<td>0.38 ± 0.13</td>
</tr>
<tr>
<td>10 mM PO₄ buffer</td>
<td>7</td>
<td>&lt;0.25</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are the average of triplicate samples +/- 1 standard deviation unless otherwise indicated.
*Value includes both detected and non-detected values. For non-detected values, one-fourth of the PQL was used in calculations.
^Values are the average of duplicate samples +/- 1 standard deviation.

### Table 2.6.5. Perchlorate levels (µg/L) in natural sunlight experiments.

<table>
<thead>
<tr>
<th></th>
<th>Light</th>
<th>Dark</th>
<th>Light</th>
<th>Dark</th>
<th>sample temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>samples on ice</td>
<td>0.37 ± 0.03</td>
<td>0.35 ± 0.04</td>
<td>0.39 ± 0.07</td>
<td>0.39 ± 0.02</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>samples at ambient temperature</td>
<td>0.45 ± 0.08</td>
<td>0.66 ± 0.15</td>
<td>0.60 ± 0.05</td>
<td>0.57 ± 0.04</td>
<td>39 ± 9</td>
</tr>
</tbody>
</table>

Perchlorate values are the average of duplicate values +/- one standard deviation.

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Figure 2.6.1. Results of MCD assays. Decreases in A278 indicate the loss of monochlorodimedone as it is converted to dichlorodimedone (Hager et al., 1966).
2.6.4 Perchlorate Formation under Nitrifying Conditions

2.6.4.1 Background
The potential for ClO₄⁻ generation by nitrifying bacteria was tested in a series of laboratory studies. The goal was to determine if ClO₄⁻ could be produced aerobically under nitrifying conditions from ClO⁻, ClO₂⁻, or ClO₃⁻ as precursors (i.e., like NO₃⁻ is produced from NH₄ and NO₂⁻ by nitrifying bacteria).

2.6.4.2 Material and Methods
Enrichment cultures for nitrifiers were obtained from sewage sludge (Ewing, NJ wastewater treatment plant), West Texas surface soils, and a freshwater fish tank. One gram of soil or 1 mL of sludge/water was inoculated into 100 mL of Winogradsky’s medium (with NH₄ from (NH₄)₂SO₄ as a sole N source) designed for isolation of nitrifiers (Atlas, 1995). Periodically, samples were tested for NO₂⁻ and NO₃⁻ levels via colorimetric test strips. After 3 days of incubation, colorimetric analysis indicated the formation of NO₂⁻ and NO₃⁻ in the flask containing sewage sludge. After six days of incubation, colorimetric analysis indicated depletion of NO₂⁻ and formation of NO₃⁻ in this same flask. Nitrate production continued to increase between six and ten days of incubation. This culture was passed into fresh Winogradsky’s medium, and production of NO₂⁻ and NO₃⁻ again was observed within 10 days of passing. The culture was passed a third and fourth time, with the same results. Nitrite and nitrate formation also occurred in samples from West Texas soils and fish tank water.

Based on the results from the nitrification enrichments, the sewage sludge enrichment in Winogradsky’s medium was selected for use as the inoculum for ClO₄⁻ formation studies. Rates and extents of NO₃⁻ formation were highest in this media. The Winogradsky’s medium was supplemented with 2 mg/L ClO₃⁻, 2mg/L ClO₂⁻, 2 mg/L ClO⁻, or no chlorinated compounds (i.e., unsupplemented); chloride-free Winogradsky’s medium (i.e., Winogradsky’s medium made as described above except that sodium chloride was omitted); and NH₄-free Winogradsky’s medium (i.e., Winogradsky’s medium made as described above except that (NH₄)₂SO₄ was omitted) supplemented with 2 mg/L ClO₃⁻, 2mg/L ClO₂⁻, 2 mg/L ClO⁻, or unsupplemented. Fifty mL of each media was placed into a 125 mL flask. Prior to inoculation, 10 mL aliquots were removed and filtered through a 0.2 micron syringe filter for analysis of anions (media baseline) (Cl⁻, NO₂⁻ (as N), SO₄²⁻, NO₃⁻ (as N), ClO₃⁻, PO₄³⁻ and ClO₂⁻) via EPA Method 300.0.

2.6.4.3 Results and Discussion
Significant NO₃⁻ formation was observed in unamended Winogradsky’s medium, medium supplemented with 2 mg/L ClO₃⁻, and Cl⁻ - free medium. In these three treatments, NO₃⁻ levels increased from ~ 45 mg/L (± 0.6; n=3) to 287 mg/L (± 2; n=3) in two weeks, confirming that nitrification was occurring. Low-level NO₃⁻ formation was also observed in NH₄-free Winogradsky’s medium supplemented with 2 mg/L ClO₃⁻ or 2 mg/L ClO₂⁻; in these treatments

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NO$_3^-$ concentrations increased from ~ 46 mg/L ($\pm$ 0.6, $n=2$) to 71 mg/L ($\pm$ 0.4, $n=2$) in two weeks. Interestingly, ClO$_3^-$ was degraded below detectable limits (less than 0.5 mg/L) under aerobic conditions within 2 weeks in Winogradsky’s medium. However, ClO$_4^-$ was not detected in these samples over the course of the study (ClO$_4^-$ detection limit was 25 µg/L). ClO$_2^-$ concentrations were reduced to 0.35 mg/L within 2 weeks in Winogradsky’s medium supplemented with ClO$_2^-$, but again ClO$_4^-$ was not detected.

The loss of ClO$_3^-$ under aerobic, nitrifying conditions is interesting. The only chlorine oxyanion that is more oxidized than ClO$_3^-$ is ClO$_4^-$, but no ClO$_4^-$ was generated during the reaction. It is possible that the loss of ClO$_3^-$ in the system was reductive (i.e., the ClO$_3^-$ was reduced to chloride), and coupled to the oxidation of NH$_4$ or NO$_2^-$. A similar process was recently proposed for the bacterium Candidatus Nitrospira defluvii (Maixner et al., 2008), when this nitrifying organism was observed to contain the gene chlorite dismutase, which would serve to dismutate (to Cl$^-$ and O) any ClO$_2^-$ formed during ClO$_3^-$ reduction, and thus mitigate toxicity of this product. Aerobic destruction of ClO$_3^-$ may be important for understanding the mass balance of chlorine oxyanions (e.g., concentrations of ClO$_3^-$ vs ClO$_4^-$) in arid environments if this process occurs routinely. However, the absence of any ClO$_4^-$ generation suggests that nitrifying strains do not produce ClO$_4^-$ from Cl$^-$, ClO$_2^-$ or ClO$_3^-$, at least in the enrichments tested during these studies.
2.6.5 Perchlorate Formation Tests Using *Starkeya novella* and West Texas Soil Enrichments

2.6.5.1 Background
The bacterial strain *Starkeya novella* (formerly *Thiobacillus novellus*) has been shown to oxidize thiosulfate (Kappler et al, 2001) via a Cytochrome c oxidoreductase that catalyzes the direct oxidation of sulfite to sulfate (Kappler et al, 2000). Based on the ability of this strain to oxidize Na$_2$S$_2$O$_3$ to SO$_4$, we tested *Starkeya novella* ATCC 8093 and unidentified strain TSP69, a strain enriched and isolated in our lab from West Texas soil, for the ability to oxidize ClO$_3^-$ to ClO$_4^-$. 

2.6.5.2 Bacterial Strains and Culture Conditions
*Starkeya novella* ATCC # 8093 was purchased from the American Type Culture Collection (Manassas, VA), and was cultured in DSMZ Medium 69: *Thiobacillus novellus* medium (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium_69.pdf). Unidentified strain TSP69 was isolated from West Texas soil inoculated and serially passed into DSMZ Medium 69 (Atlas, 1995).

Several variations of this medium were used, including unadjusted/neutral pH, as well as substituting sodium sulfite or sodium phosphite for sodium thiosulfate. Cultures were given additional thiosulfate, sulfite, or phosphite up to three times per week. After 2 passes into fresh media, cultures were streaked onto agar plates. All cultures, regardless of pH or O$_3$-containing compound, were shown to be pure cultures that were morphologically the same. One culture, grown on sodium thiosulfate at neutral pH, was chosen for further study.

2.6.5.3 Perchlorate Formation Study Methods and Procedures
*Media.* The variations of DSMZ Medium 69 used in this study can be seen in Table 2.6.6.
Table 2.6.6. Variations of DSMZ Medium 69 used in the perchlorate formation studies.

<table>
<thead>
<tr>
<th>Variation #</th>
<th>O₃-containing compound (5 mM)</th>
<th>Perchlorate precursor (0.1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sodium thiosulfate</td>
<td>Chlorate (as sodium chlorate)</td>
</tr>
<tr>
<td>2</td>
<td>Sodium thiosulfate</td>
<td>Chlorite (as sodium chlorite)</td>
</tr>
<tr>
<td>3</td>
<td>Sodium sulfite</td>
<td>Chlorate (as sodium chlorate)</td>
</tr>
<tr>
<td>4</td>
<td>Sodium sulfite</td>
<td>Chlorite (as sodium chlorite)</td>
</tr>
<tr>
<td>5</td>
<td>Sodium phosphite</td>
<td>Chlorate (as sodium chlorate)</td>
</tr>
<tr>
<td>6</td>
<td>Sodium phosphite</td>
<td>Chlorite (as sodium chlorite)</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>Chlorate (as sodium chlorate)</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>Chlorite (as sodium chlorite)</td>
</tr>
<tr>
<td>9</td>
<td>Sodium thiosulfate</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>Sodium sulfite</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>Sodium phosphite</td>
<td>None</td>
</tr>
</tbody>
</table>

One flask of each media type was inoculated with either *Starkeya novella* or strain TSP69, or was left uninoculated to serve as a no cell control. Flasks were placed in an incubator/shaker operating at room temperature (approximately 22-23°C). Flasks were sampled weekly as follows. Flasks were removed from the shaker. Ten mL was removed aseptically and filtered through a 0.2 μm filter for perchlorate analysis. The volume removed was then replaced with fresh sterile medium and the flasks were replaced in the incubator. All samples were refrigerated until being shipped on ice to Texas Tech for ClO₄⁻ analysis as previously described.

2.6.5.4 Results and Discussion

Cultures grown on thiosulfate exhibited the most growth, followed by those grown on sulfite, as evidenced by visual inspection. Cultures grown on phosphite exhibited significantly less growth than those grown on the sulfur-containing compounds. Samples were chosen for ClO₄⁻ analysis based on the amount of growth observed (i.e., samples from flasks exhibiting the most growth were chosen for further analysis). Analysis was also performed on the appropriate controls. Results of ClO₄⁻ analysis are provided in Table 2.6.7. The data showed no significantly enhanced ClO₄⁻ production in any set of live samples compared to uninoculated controls.

To confirm these results, (i.e., that no significant ClO₄⁻ production was observed), selected culture conditions were repeated in a second experiment. Variations # 4, 5, 6, 10, 11, and 12 were set up in duplicate, and variations 22-30 were set up singularly as controls. Cultures were incubated, sampled, and fed as described above, except that sampling and feeding was performed every 3 weeks (i.e., at T=3 weeks and 6 weeks). No ClO₄⁻ generation was documented.
Table 2.6.7. Perchlorate concentrations (µg/L) in studies with *Starkeya novella* and TSP69.

<table>
<thead>
<tr>
<th>Time (date)</th>
<th>Media components</th>
<th>Inoculants</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>TSP 69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/15/2012</td>
<td>7</td>
<td>X</td>
</tr>
<tr>
<td>2/22/2012</td>
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<td>X</td>
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<tr>
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<td>X</td>
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<tr>
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<td>X</td>
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<td>14</td>
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<tr>
<td>3/8/2012</td>
<td>29</td>
<td>X</td>
</tr>
</tbody>
</table>

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2.7 Evaluate the Accumulation of ClO$_4^-$ in Plants and their Role in Ozone-Mediated Production of ClO$_4^-$

2.7.1 ClO$_4^-$ Accumulation at Environmentally-Relevant O$_3$ Concentrations.

2.7.1.1 Background
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., 2011a,b; Booker et al., 2009; USEPA, 2012). 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 Cl$^-$ 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.
2.7.1.2 Materials and Methods

Plant Material

We chose 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 x 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.

Ozone Exposure

Plants were exposed to environmentally relevant O$_3$ concentrations (12 hour 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 O₃ monitor (ThermoElectron Model 41C), with other CSTRs controlled by ratio of O₃ 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 O₃ concentration was introduced at one complete air exchange per minute.

**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 ClO₄⁻ analysis.

Soil samples were extracted using Milli-Q water at a 2:1 mass ratio (water: soil) by shaking for 24 hours. The samples were centrifuged for 10 minutes 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 ClO₄⁻), and an extraction blank (DDI water only). The moisture content of parallel samples was determined by drying at 105 °C for 24 hours. The final filtered extract was analyzed for major anions and ClO₄⁻.

Plant leaf samples were pre-dried (105° C for 12 hrs) and approximately 1 gm 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 hr (water bath temperature ~ 99 °C) and centrifuged at 5000 rpm for 5 minutes. 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 hrs. The suspension was then re-centrifuged at 5000 rpm for 5 minutes, 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 ClO₄⁻.
Perchlorate in the resulting solutions was quantified by IC-MS/MS at Texas Tech University as described in previous sections of this report. 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\(^{-1}\)).

**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 hour 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 two hour 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.

**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.

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. 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.
2.7.1.3 Results

Sources of Perchlorate

The plants were irrigated with water containing very little ClO₄⁻ (Table 2.7.1). Similarly, the commercial potting mix exhibited relatively low ClO₄⁻ content. The commercial fertilizer applied to all species contained substantial amounts of ClO₄⁻ on a dry weight basis (Table 2.7.1), but only 3.6 µg ClO₄⁻ 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 ClO₄⁻ from the fertilizer that was applied twice weekly, and an additional 2.6 µg ClO₄⁻ in irrigation water applied daily. These provided the principal source for ClO₄⁻ accumulation from the growth medium (Table 2.7.1).

Perchlorate Accumulation

Large interspecific differences were observed in foliar concentrations of ClO₄⁻ averaged across all O₃ exposures (Figure 2.7.1; Table 2.7.2). These appear to reflect physiological differences in uptake or exclusion of ClO₄⁻ present in the growth medium. Spinach accumulated approximately 700 µg (kg dry wt)⁻¹, the highest level observed, while sugarcane accumulated less than 100 µg kg⁻¹. Other species were intermediate, with young leaf contents lying generally between 150-250 µg kg⁻¹. On average, plants accumulated about 7% of the applied ClO₄⁻ over their lifespan, much of the remainder being lost to drainage.

The results were similar for older, senescing leaves (Table 2.7.3) although ClO₄⁻ was accumulated to much higher levels in these leaves. Young leaves of broccoli exhibited contents near 400 µg kg⁻¹ (Table 2.7.2) whereas older leaves averaged slightly over 1000 µg kg⁻¹ (Table 2.7.3). For sugarcane, the corresponding values were about 50 µg kg⁻¹ for young leaves (Table 2.7.2) but >100 µg kg⁻¹ for older leaves (Table 2.7.3). Averaged over all species, older leaves accumulated more than twice the ClO₄⁻ as younger leaves (>500 µg kg⁻¹ vs. <250 µg kg⁻¹).

Effect of Ozone Exposure on Perchlorate Accumulation

There was no consistent effect of O₃ exposure on foliar content of ClO₄⁻ in young leaves. Five of nine species exhibited a decline in ClO₄⁻ content with increasing O₃ exposure while for the remaining four species ClO₄⁻ content increased with O₃ exposure. In no species was this response significant (Table 2.7.2) and even with the additional statistical power of combining all species (n=68), the mean response across all species was not significantly related to O₃ exposure (Table 2.7.2). The same absence of relationship with O₃ exposure was observed in older leaves, despite the greater overall accumulation of ClO₄⁻ (Table 2.7.3).

Basal accumulation of ClO₄⁻ at low O₃ was not predictive of the effect of elevated O₃, on ClO₄⁻ in young leaves, whether significant or not (Figure 2.7.2; Table 2.7.4). This was the case for absolute changes in ClO₄⁻ between low and moderate O₃ and between low and higher O₃
exposures (Figure 2.7.2A; triangles, squares, respectively). The relationship was not improved by normalization of the values associated with O₃ exposure by basal ClO₄⁻ content (Figure 2.7.2B).

We considered whether O₃-induced foliar biosynthesis of ClO₄⁻ might occur, yet not be reflected in leaf contents due to transport to the rhizosphere. However, there was no relationship between ClO₄⁻ in the top level of the potting medium and basal accumulation of ClO₄⁻ at 4 ppb O₃ (Table 2.7.4). Similarly, there was no significant relationship between O₃ exposure of plant and potting medium and surface content of ClO₄⁻ (Table 2.7.4). However, a multiple range test of ClO₄⁻ in the surface layer of the growth medium of unused potting medium, potting medium exposed at 4 ppb O₃, and potting medium exposed at 114 ppb O₃ (Table 2.7.5) suggested a positive association between ClO₄⁻ and O₃. The potting medium exposed to the highest and lowest O₃ concentrations did not differ in ClO₄⁻ content, and that exposed to the lowest O₃ did not differ from the unused medium. However, there was a significant difference between unused potting medium and that exposed to the highest O₃ concentration.
Table 2.7.1. ClO₄⁻ content of unused irrigation water, potting medium, and fertilizer.

<table>
<thead>
<tr>
<th>Material</th>
<th>n</th>
<th>Perchlorate Content</th>
<th>S.E.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation Water</td>
<td>11</td>
<td>0.61 (µg L⁻¹)</td>
<td>± 0.053</td>
<td>0.33 – 0.81</td>
</tr>
<tr>
<td>Potting Medium</td>
<td>12</td>
<td>3.78 (µg (kg dry wt)⁻¹)</td>
<td>± 0.72</td>
<td>0.43-6.41</td>
</tr>
<tr>
<td>Fertilizer (granular)</td>
<td>6</td>
<td>1270 (µg (kg dry wt)⁻¹)</td>
<td>± 89.3</td>
<td>1090-1700</td>
</tr>
<tr>
<td>(asapplied)</td>
<td></td>
<td>3.55 (µg L⁻¹)</td>
<td>± 0.25</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7.2. Perchlorate content (µg kg\(^{-1}\)) of young leaves of a range of crop species as a function of O\(_3\) exposure (ppb, 12 hr 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>O(_3) Exposure (ppb)</th>
<th>Perchlorate Content (µg kg(^{-1}))</th>
<th>S.E.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bush Bean</td>
<td>6</td>
<td>4</td>
<td>155 ± 31.3</td>
<td></td>
<td>72.1-294</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>59</td>
<td>160 ± 19.0</td>
<td></td>
<td>110-225</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>114</td>
<td>204 ± 15.4</td>
<td></td>
<td>130-236</td>
</tr>
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<td>Broccoli</td>
<td>9</td>
<td>4</td>
<td>380 ± 41.4</td>
<td></td>
<td>234-653</td>
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<tr>
<td></td>
<td>7</td>
<td>59</td>
<td>349 ± 75.0</td>
<td></td>
<td>42.1-672</td>
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<tr>
<td></td>
<td>9</td>
<td>114</td>
<td>528 ± 86.1</td>
<td></td>
<td>68.3-927</td>
</tr>
<tr>
<td>Lettuce</td>
<td>9</td>
<td>4</td>
<td>223 ± 89.0</td>
<td></td>
<td>65.8-926</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>59</td>
<td>145 ± 26.3</td>
<td></td>
<td>56.2-279</td>
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<tr>
<td></td>
<td>9</td>
<td>114</td>
<td>151 ± 20.1</td>
<td></td>
<td>81.3-250</td>
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<td>Maize</td>
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<td>173 ± 54.1</td>
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<td></td>
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<td>59</td>
<td>85 ± 10.2</td>
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</tr>
<tr>
<td></td>
<td>6</td>
<td>114</td>
<td>123 ± 38.3</td>
<td></td>
<td>23.9-295</td>
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<tr>
<td>Pima Cotton</td>
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<td>4</td>
<td>290 ± 40.2</td>
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<td>18</td>
<td>59</td>
<td>218 ± 24.8</td>
<td></td>
<td>75.5-416</td>
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<tr>
<td></td>
<td>18</td>
<td>114</td>
<td>218 ± 36.3</td>
<td></td>
<td>58.6-590</td>
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<td>Sorghum</td>
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<td>177 ± 38.4</td>
<td></td>
<td>87.5-328</td>
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<tr>
<td></td>
<td>6</td>
<td>59</td>
<td>182 ± 20.6</td>
<td></td>
<td>128-267</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>114</td>
<td>369 ± 110.3</td>
<td></td>
<td>84.2-598</td>
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<tr>
<td>Soybean</td>
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<td>4</td>
<td>222 ± 39.8</td>
<td></td>
<td>136-366</td>
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<td>6</td>
<td>59</td>
<td>218 ± 16.8</td>
<td></td>
<td>164-284</td>
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<tr>
<td></td>
<td>6</td>
<td>114</td>
<td>186 ± 24.7</td>
<td></td>
<td>123-294</td>
</tr>
<tr>
<td>Spinach</td>
<td>3</td>
<td>4</td>
<td>766 ± 84.5</td>
<td></td>
<td>670-934</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>59</td>
<td>568 ± 110.6</td>
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<td>458-679</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>686 ± 31.8</td>
<td></td>
<td>622-719</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>6</td>
<td>4</td>
<td>42.5 ± 6.2</td>
<td></td>
<td>30.6-71.4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>59</td>
<td>61.0 ± 19.0</td>
<td></td>
<td>6.67-132</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>114</td>
<td>54.6 ± 15.3</td>
<td></td>
<td>15.0-122</td>
</tr>
<tr>
<td>All Species</td>
<td>68</td>
<td>4</td>
<td>255 ± 24.8</td>
<td></td>
<td>15.00-934</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>59</td>
<td>199 ± 17.0</td>
<td></td>
<td>6.67-679</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>114</td>
<td>255 ± 26.1</td>
<td></td>
<td>15.0-927</td>
</tr>
</tbody>
</table>
Table 2.7.3. Perchlorate content of older, senescing leaves of a range of crop species as a function of O₃ exposure (ppb, 12 hr mean) sampled at the time of final harvest. There were no significant differences between O₃ exposures (P < 0.05) within any species, nor within the pooled data.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>O₃ Exposure (ppb)</th>
<th>Perchlorate Content (µg kg⁻¹)</th>
<th>S.E.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>9</td>
<td>4</td>
<td>1730 ± 970</td>
<td>312-9450</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>59</td>
<td>765 ± 123</td>
<td>423-1430</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>114</td>
<td>945 ± 170</td>
<td>417-1960</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>2</td>
<td>4</td>
<td>337 ± 188</td>
<td>150-525</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>430 ± 171</td>
<td>165-750</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>114</td>
<td>319 ± 62.5</td>
<td>188-750</td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>6</td>
<td>4</td>
<td>87.0 ± 19.8</td>
<td>51.5-184</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>59</td>
<td>85.1 ± 14.0</td>
<td>17.4-110</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>114</td>
<td>77.4 ± 11.6</td>
<td>54.9-120</td>
<td></td>
</tr>
<tr>
<td>Pima Cotton</td>
<td>12</td>
<td>4</td>
<td>435 ± 133</td>
<td>97.5-1320</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>59</td>
<td>352 ± 74.6</td>
<td>78.1-1280</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>114</td>
<td>549 ± 170</td>
<td>70.3-2330</td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>3</td>
<td>4</td>
<td>238 ± 11.8</td>
<td>214-250</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>527 ± 166.4</td>
<td>348-860</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>463 ± 77.3</td>
<td>375-617</td>
<td></td>
</tr>
<tr>
<td>Sugarcane</td>
<td>3</td>
<td>4</td>
<td>131 ± 22.7</td>
<td>104-176</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>85.7 ± 16.0</td>
<td>58.4-114</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>114</td>
<td>95.4 ± 7.94</td>
<td>72.1-125</td>
<td></td>
</tr>
<tr>
<td>All Species</td>
<td>35</td>
<td>4</td>
<td>660 ± 267</td>
<td>51.5-9450</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>59</td>
<td>394 ± 55.2</td>
<td>17.4-1432</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>114</td>
<td>462 ± 78.7</td>
<td>54.9-2330</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7.4. 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 O₃ exposure (ppb, 12 hr mean). There were no significant differences between O₃ exposures (P < 0.05) within any species, nor within the pooled data.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>O₃ Exposure (ppb)</th>
<th>Perchlorate Content (µg kg$^{-1}$)</th>
<th>S.E.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>6</td>
<td>4</td>
<td>5.68 ± 0.40</td>
<td>5.00-7.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>59</td>
<td>6.09 ± 0.26</td>
<td>5.38-7.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>114</td>
<td>6.2 ± 0.39</td>
<td>4.62-7.31</td>
<td></td>
</tr>
<tr>
<td>Bush Bean</td>
<td>3</td>
<td>4</td>
<td>6.98 ± 0.08</td>
<td>6.88-7.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>6.53 ± 0.2</td>
<td>6.15-6.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>6.14 ± 0.25</td>
<td>5.88-6.64</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>6</td>
<td>4</td>
<td>6.13 ± 0.27</td>
<td>5.49-7.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>59</td>
<td>5.7 ± 0.21</td>
<td>5.07-6.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>114</td>
<td>7.32 ± 1.08</td>
<td>6.00-12.63</td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>3</td>
<td>4</td>
<td>0.51 ± 0.05</td>
<td>0.43-0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>2.8 ± 0.00</td>
<td>2.80-2.80</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>0.44 ± 0.06</td>
<td>0.37-0.55</td>
<td></td>
</tr>
<tr>
<td>Pima Cotton</td>
<td>9</td>
<td>4</td>
<td>1.38 ± 0.29</td>
<td>0.65-2.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>59</td>
<td>3.44 ± 2.02</td>
<td>0.69-17.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>114</td>
<td>4.49 ± 2.03</td>
<td>1.03-18.6</td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>3</td>
<td>4</td>
<td>7.79 ± 0.88</td>
<td>6.70-9.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>7.16 ± 0.09</td>
<td>6.98-7.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>8.65 ± 1.32</td>
<td>7.32-11.3</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>3</td>
<td>4</td>
<td>6.96 ± 0.31</td>
<td>6.47-7.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>6.65 ± 0.61</td>
<td>5.54-7.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>6.41 ± 0.25</td>
<td>5.95-6.82</td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>3</td>
<td>4</td>
<td>5.73 ± 0.21</td>
<td>5.34-6.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>6.05 ± 0.87</td>
<td>4.59-7.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>6.26 ± 0.17</td>
<td>5.93-6.49</td>
<td></td>
</tr>
<tr>
<td>Sugarcane</td>
<td>3</td>
<td>4</td>
<td>10.7 ± 3.18</td>
<td>4.53-15.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>59</td>
<td>7.55 ± 0.43</td>
<td>6.81-8.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114</td>
<td>9.31 ± 1.83</td>
<td>5.70-11.6</td>
<td></td>
</tr>
<tr>
<td>All Species</td>
<td>39</td>
<td>4</td>
<td>5.11 ± 0.53</td>
<td>0.43-15.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>59</td>
<td>5.63 ± 0.50</td>
<td>0.69-17.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>114</td>
<td>5.98 ± 0.61</td>
<td>0.37-18.6</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7.5. 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 hr mean). Means followed by different letters were significantly different (P < 0.05).

<table>
<thead>
<tr>
<th>O(_3) Exposure (ppb)</th>
<th>n</th>
<th>Perchlorate Content (µg kg(^{-1}))</th>
<th>S.E.</th>
<th>Range (µg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>na</td>
<td>12</td>
<td>3.78(^a)</td>
<td>± 0.72</td>
<td>0.43-6.41</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>5.11(^{ab})</td>
<td>± 0.53</td>
<td>0.43-15.1</td>
</tr>
<tr>
<td>114</td>
<td>39</td>
<td>5.98(^b)</td>
<td>± 0.61</td>
<td>0.37-18.6</td>
</tr>
</tbody>
</table>
Figure 2.7.1. Inter-specific differences in perchlorate content (µg (kg dry wt)$^{-1}$) of young ones 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.
Figure 2.7.2. 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.
**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. 2.7.3) revealed a non-significant relationship between normalized ClO$_4^-$ and O$_3$ exposure (Fig. 2.7.3A), consistent with the results of the ANOVA with young leaves (Table 2.7.2). The relationship was not improved when ClO$_4^-$ was considered as a function of O$_3$ dose (Fig. 2.7.3B).
Figure 2.7.3. There was no significant relationship between O₃ exposure (A; $r^2 = 0.0034$) or O₃ dose (cumulative flux; B; $r^2=0.0018$) and ClO₄⁻ content of young leaves normalized by the median ClO₄⁻ content of each species shown in Figure 2.7.1 for which O₃ flux data were available.
2.7.1.4 Discussion

**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*, 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 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., 2006).

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.

**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., 2008a; 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$_4^-$.

**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\textsuperscript{-} in the irrigation water or fertilizer were exposed directly to O\textsubscript{3} in the CSTRs. There was some indication that direct exposure of the growth medium to the highest O\textsubscript{3} concentration may have led to accumulation of ClO\textsubscript{4}\textsuperscript{-} in the surface layer. Averaged over all species there was a modest but non-significant relationship with increasing O\textsubscript{3} exposure, but a significant difference between unused potting medium and medium exposed to the highest O\textsubscript{3}. This suggests that ClO\textsubscript{4}\textsuperscript{-} content in the soil surface may increase with exposure to O\textsubscript{3}, presumably due to oxidation of Cl\textsuperscript{-} in the soil, as demonstrated experimentally at higher O\textsubscript{3} concentrations with Cl\textsuperscript{-} coated sand (Kang et al., 2008) and soil (Dasgupta et al., 2005). Our results were observed without artificial enhancement of the Cl\textsuperscript{-} content, at near ambient O\textsubscript{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\textsubscript{4}\textsuperscript{-} present in the environment.

The clear absence of O\textsubscript{3}-sensitivity of ClO\textsubscript{4}\textsuperscript{-} 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\textsubscript{4}\textsuperscript{-} 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\textsubscript{4}\textsuperscript{-} 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\textsuperscript{-1}), while roots and stems accumulated only 100-150 µg (kg dwt\textsuperscript{-1}) (Tan et al., 2006). Neither exudation to non-contaminated media nor reductive metabolism are significant sinks for phytoaccumulated ClO\textsubscript{4}\textsuperscript{-}, (van Aken and Schnoor, 2002), though some metabolites were detected in Populus.
2.7.1.5 Conclusions
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.
2.7.2 ClO₄⁻ Accumulation at Elevated O₃ Concentrations

2.7.2.1 Background

In the previous Section (2.7.1), we demonstrated that low to moderate concentrations of O₃ (0-114 nL L⁻¹, 12 hr mean) did not increase foliar ClO₄⁻, despite large interspecific differences in ClO₄⁻ uptake from the growth medium. However, these previous data did not disprove the hypothesis that much higher concentrations of O₃, such as that observed during stratosphere-troposphere folding events or during O₃ episodes in emerging mega-cities, might stimulate O₃ synthesis or accumulation in plants. Here we test the hypothesis that exposure of plants to environmentally plausible, but very high, concentrations of O₃ may lead to accumulation of ClO₄⁻ in plant leaf tissue or in the soil surrounding the plants.

2.7.2.2 Material and Methods

Plant Material

We examined 5 crops: the C₃ dicotyledonous species, soybean (*Glycine max* cv. Disoy; Ferry Morse Seed Co., Fulton KY), Pima cotton (*Gossypium barbadense* 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), and the C₄ monocotyledonous species, sorghum (*Sorghum bicolor* cv. 4662, Pioneer Seed Co., Johnston IA), and maize (*Zea mays* cv. Golden Cross Bantam (hybrid); Ferry Morse Seed Co., Fulton KY). Some of these cultivars are known to be relatively sensitive to O₃, while the sensitivity of others has not been evaluated.

Seeds were planted in moist potting mix (Earthgro Potting Soil; Scotts Company, Marysville, OH) and subsequently thinned to 1 plant per pot (10 cm x 10 cm x 13 cm). Plants were grown under greenhouse conditions at Kearney Research and Extension Center (103 msl; 36.598 N 119.503 W) with daily drip irrigation and twice weekly application of a complete fertilizer solution (2.9 g L⁻¹, Miracle Gro, Scotts Miracle-Gro Products Inc., Port Washington, NY). These plants were exposed to relatively low amounts of ClO₄⁻ in their growth environment. The ClO₄⁻ content of the fertilizer was 3.55 ± 0.25 µg L⁻¹, and that of the potting soil and irrigation water were 3.77 ± 0.72 µg and 0.61 ± 0.05 µg L⁻¹ (kg dry wt)⁻¹, respectively (Section 2.6; Grantz et al., 2014). Pots retained 68.9 mL of solution against drainage so that over 9 weeks of growth, each plant had access to approximately 7 µg ClO₄⁻, the principal substrate for ClO₄⁻ accumulation.

Ozone Exposure

Plants were grown from germination through harvest in Teflon greenhouse exposure chambers (CSTRs; Section 2.7.1; Heck et al., 1978; Grantz et al., 2010; 2014). Growth temperature was 15-30 °C, illumination with natural sunlight was about 300 µmol m⁻² s⁻¹ PPFD at plant level, reflecting shading by both the greenhouse structure and the CSTR. Air with appropriate O₃ concentrations (12 hour means nominally 4, 102, and 204 nL L⁻¹; daily maxima near solar noon
of 4, 160 and 320 nL L\(^{-1}\)) was introduced at one complete air exchange per minute into each CSTR.

O\(_3\) was produced by corona discharge (Model SGC-11, Pacific O\(_3\) Technology, Brentwood, CA) from a feedstock of purified oxygen (Series ATF-15, Model 1242, SeQual Technologies Inc., San Diego CA). O\(_3\) concentration followed a half-sine wave during daylight hours, 7 days week\(^{-1}\), with voltage to the O\(_3\) generator regulated by feedback from the exit stream of a master CSTR using an ozone monitor (Model 41C; Thermo Electron Corp.; Franklin MA, USA) calibrated against an O\(_3\) calibration unit (Model 306; 2B Technologies, Boulder, CO, USA). The remaining CSTRs were controlled proportionally (Grantz et al., 2010) and monitored independently (Model 41C).

O\(_3\) flux into the leaves was determined from bi-hourly measurements of stomatal conductance of the youngest fully expanded leaf and of the leaf two insertion levels older, using a porometer (AP4; Delta T Devices, Cambridge UK). Values are means of the two leaves, replicated in 3 CSTRs at each ozone concentration and over 2 runs with different sets of plants (n = 6). To calculate O\(_3\) flux, conductance was converted from water vapor to O\(_3\) using the ratio of diffusivities (Massman and Grantz, 1995; Massman, 1998), and multiplied by mean O\(_3\) concentration during the 2 hour period centered on the conductance measurement. O\(_3\) dose was calculated as flux summed over all daylight hours from germination to harvest.
Perchlorate Determination

All healthy leaves and the surface 5 cm of potting soil were collected at 9 weeks after planting and immediately frozen at -20 °C for shipping on dry ice to Texas Tech University for determination of ClO$_4^-$ by ion chromatograph-tandem mass spectrometer (IC-MS/MS) as previously described in this report.

Samples were dried to constant weight (105° C; 12 hrs) and 1 g placed in a centrifuge tube with 25 mL of Milli-Q water, immersed in boiling water for 1 h, then centrifuged at 5000 rpm for 5 minutes. The supernatant (2 ml) was added to 1.0 ± 0.1 g of activated alumina, and diluted with 18 mL of DDI water, held at 3 °C for 8 h, then centrifuged at 5000 rpm for 5 minutes. The supernatant was filtered (0.2 micron), passed through a pre-cleaned and activated OnGuard® RP cartridge (Dionex Corporation; Sunnyvale CA), then diluted.

All extraction sets were accompanied by an extraction duplicate, an extraction spike (sample + known amount of added ClO$_4^-$), and an extraction blank (DDI water only). Samples were spiked with an oxygen-isotope ($^{18}$O) labeled ClO$_4^-$ internal standard, then loaded into a 25 µL pre-concentrating loop. This was connected to the IC-MS/MS, with a GP50 pump, CD25 conductivity detector, AS40 automated sampler and Dionex IonPac AS16 (250 X 2 mm) analytical column, followed by an Applied Biosystems - MDS SCIEX API 2000® triple quadrupole mass spectrometer with a Turbo-IonSpray™ source. The eluent was 45 mM sodium hydroxide (NaOH) followed by 90% acetonitrile as a post-column solvent (both at 0.3 ml min$^{-1}$). The method detection limit (MDL) was 0.01 µM ClO$_4^-$. 

Statistical Analysis

CSTRs were arrayed in three blocks parallel to windows and cooling fans, to isolate location effects. One CSTR per block was exposed to each of the three O$_3$ concentrations. Two runs were conducted with each species (n=6). The individual CSTR was taken as the unit of replication. Block and run were not significant and were pooled.

The relationship between ClO$_4^-$ and O$_3$ exposure was evaluated independently for each species and for all species pooled, by regression analysis (SAS v. 9.3; PROC REG; SAS Institute, 2002). The relationship between ClO$_4^-$ and O$_3$ dose was evaluated similarly, using both native ClO$_4^-$ data and ClO$_4^-$ data normalized within each species by the median value of ClO$_4^-$ determined in each species over all O$_3$ exposures.

Basal ClO$_4^-$ content of all species at low O$_3$ concentration was subjected to independent analysis by ANOVA (PROC GLM and PROC MEANS), with means separation by Duncan’s Multiple
Significant differences were identified at \( P < 0.05 \). Means are presented with standard errors of the means.

2.7.2.3 Results and Discussion

**Species Differences in Perchlorate Uptake**

There was considerable interspecific variability in the capacity for accumulation of ClO\(_4^–\) from the rhizosphere (Table 2.7.6). The large capacity to accumulate ClO\(_4^–\) from the environment in some species has been observed in other systems (Grantz et al., 2014; Jackson et al., 2005; Nzengung et al., 1999; Tan et al., 2004, 2005; Urbansky et al., 2000, 2000a; van Aken and Schnoor, 2002; Yu et al., 2004; Yang and Her, 2011). This capacity, in species including cottonwood (*Populus* spp. hybrids), *Eucalyptus cineria*, willow (*Salix nigra*), tamarisk (*Tamarix ramosissima*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), and soybean (*Glycine max*), among others, has implications for bioremediation of contaminated soils and aquifers. Among the species examined here, foliar contents of ClO\(_4^–\) at low O\(_3\) ranged from about 130 ± 16 µg (kg dry wt\(^{-1}\)) (range 87 – 205 µg (kg dry wt\(^{-1}\)) in Pima cotton to over 500 ± 191 µg (kg dry wt\(^{-1}\)) (range 252 – 1469 µg (kg dry wt\(^{-1}\)) in young leaves of soybean (Table 2.7.6). In our previous study described in Section 2.7.1, spinach (*Spinacia oleracea*) accumulated over 700 µg (kg dry wt\(^{-1}\)), while sugarcane (*Saccharum* spp., hybrid) accumulated less than 100 µg (kg dry wt\(^{-1}\)).

These species differences reflect physiological differences in uptake/exclusion of ClO\(_4^–\) present in the growth medium. In both studies, plants accumulated less than 10% of the available ClO\(_4^–\) over the entire study period, but exhibited bioconcentration factors of up to two orders of magnitude. Accumulation of ClO\(_4^–\) was greater in older than in younger leaves as described in Section 2.7.1, reflecting the greater duration of uptake. ClO\(_4^–\) in the foliage is attributed to transpiration and deposition at the distributed sites of evaporation. In willow (*Salix nigra*) uptake was linear with transpiration rate (Nzengung et al., 1999) until a state of tissue saturation reduced net uptake to zero. In diverse types of lettuce (*Lactuca sativa*), the transpiring outer leaves contained an order of magnitude greater ClO\(_4^–\) than the sheltered inner leaves (Ha et al., 2013). Re-translocation of ClO\(_4^–\) has not been reported.

Root and stem tissue exhibit very low ClO\(_4^–\) contents relative to leaves, as observed in the present study (not shown; Grantz, et al., 2014) and in other species (Vogt and Jackson, 2010). In smartweed (*Polygonum* spp.), leaves accumulated up to 800 µg (kg dry wt\(^{-1}\)), while roots and stems contained less than 150 µg (kg dry wt\(^{-1}\)) (Tan et al., 2006).

Accumulation of ClO\(_4^–\) from the rhizosphere, its sequestration in older leaf cohorts, and their subsequent abscission, represents a possible mechanism of transporting and concentrating rhizosphere ClO\(_4^–\) to the soil surface (Section 2.7.1; Grantz et al., 2014). Under natural conditions and longer time frames this mechanism may be significant, but in the short term
experiments described here was not observed. Soil surface ClO₄⁻ values ranged from 6.2 to 8.4 µg (kg dry wt)⁻¹ (Table 2.7.7) at the highest O₃ exposure, similar to the values observed previously at lower O₃ (Table 2.7.4 in Section 2.7.1 and Table 2.7.7 herein). There was no relationship between plant capacity for ClO₄⁻ accumulation and the ClO₄⁻ content of the surface soil.

Table 2.7.6. Perchlorate concentration (µg (kg dry wt)⁻¹) of young leaves of contrasting crop species exposed to filtered air. Values followed by the same letter do not differ at P < 0.05.

<table>
<thead>
<tr>
<th>Species</th>
<th>O₃ Exposure (nL L⁻¹)</th>
<th>n</th>
<th>Perchlorate Content (µg (kg dry wt⁻¹))</th>
<th>S.E.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>4</td>
<td>6</td>
<td>524.54 a</td>
<td>± 190.88</td>
<td>251.90-1469.00</td>
</tr>
<tr>
<td>Bush bean</td>
<td>4</td>
<td>6</td>
<td>307.90 a,b</td>
<td>± 95.60</td>
<td>119.50-678.30</td>
</tr>
<tr>
<td>Maize</td>
<td>4</td>
<td>6</td>
<td>160.33 b</td>
<td>± 76.49</td>
<td>30.00-514.10</td>
</tr>
<tr>
<td>Sorghum</td>
<td>4</td>
<td>6</td>
<td>134.34 b</td>
<td>± 13.18</td>
<td>90.21-174.10</td>
</tr>
<tr>
<td>Pima Cotton</td>
<td>4</td>
<td>6</td>
<td>133.86 b</td>
<td>± 16.37</td>
<td>86.64-205.10</td>
</tr>
</tbody>
</table>
Table 2.7.7. Perchlorate concentration (µg (kg dry wt)$^{-1}$) of potting medium used for plant growth by contrasting crop species exposed to high O$_3$. Values followed by the same letter do not differ at P < 0.05.

<table>
<thead>
<tr>
<th>Species</th>
<th>O$_3$ Exposure (nL L$^{-1}$)</th>
<th>n</th>
<th>Perchlorate Content (µg (kg dry wt)$^{-1}$)</th>
<th>S.E.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>204</td>
<td>4</td>
<td>8.39 a</td>
<td>± 2.77</td>
<td>4.86-16.64</td>
</tr>
<tr>
<td>Bush bean</td>
<td>204</td>
<td>4</td>
<td>5.92 a</td>
<td>± 0.48</td>
<td>4.97-7.24</td>
</tr>
<tr>
<td>Maize</td>
<td>204</td>
<td>4</td>
<td>7.74 a</td>
<td>± 2.07</td>
<td>4.44-13.80</td>
</tr>
<tr>
<td>Sorghum</td>
<td>204</td>
<td>4</td>
<td>6.89 a</td>
<td>± 1.41</td>
<td>4.53-10.60</td>
</tr>
<tr>
<td>Pima Cotton</td>
<td>204</td>
<td>4</td>
<td>6.20 a</td>
<td>± 0.18</td>
<td>5.84-6.71</td>
</tr>
</tbody>
</table>
**O3 Impacts on ClO$_4^-$ Accumulation**

The soil surface and any Cl$^-$ in the irrigation water or fertilizer near the surface were exposed directly to O$_3$. Soil surface enhancement of ClO$_4^-$ could occur with increasing O$_3$ exposure due to soil surface reactions. The average soil surface ClO$_4^-$ concentration, over all species at the highest O$_3$ (204 nL L$^{-1}$), was 7.03 ± 0.70 µg (kg dry wt)$^{-1}$, significantly greater than the ClO$_4^-$ concentration of 3.77 ± 0.72 µg (kg dry wt)$^{-1}$ observed at the lowest O$_3$, and greater than that observed in the unused potting medium (Fig. 2.7.4). The unused potting mix (sampled directly from an unopened container) was non-significantly lower than that exposed to the low O$_3$, indicating that placement of the plant and soil in the CSTR, even in the absence of appreciable O$_3$, may have caused an increase in ClO$_4^-$ due to unknown heterogeneous surface reactions.

The ClO$_4^-$ concentration of surface soil increased linearly with increasing O$_3$ exposure (Fig. 2.7.4). A similar trend was observed in our previous study described in Section 2.7.1. The short exposure period in both of our experiments suggests that over longer time periods exposure of the soil surface to environmental O$_3$ could lead to environmentally significant accumulation of ClO$_4^-$. Soil ClO$_4^-$ does not appear to be attributable to retranslocation from leaves to root exudates. Such exudation of phytoaccumulated ClO$_4^-$ was not observed into ClO$_4^-$-free media in Populus (van Aken and Schnoor, 2002).

In contrast to the soil surface, there was no effect of O$_3$ exposure on foliar content of ClO$_4^-$ in young leaves of any of the 5 crop species considered (not shown). Combining the data for all species increased the sample size substantially. This analysis also revealed no significant relationship between O$_3$ exposure and foliar ClO$_4^-$ accumulation (Fig. 2.7.5).

**Stomatal Responses and O$_3$ Dose**

The use of O$_3$ concentration as a metric of O$_3$ exposure has limitations, associated with temporal shifts between stomatal opening and elevated ambient O$_3$ concentrations (Massman et al., 2000). This is addressed by use of measured or calculated uptake or flux of O$_3$ into the leaf, (i.e. O$_3$ dose) (Grunhage et al., 1997). Here, we directly measured stomatal conductance ($g_s$) over the diel timecourse, in sorghum (Fig. 2.7.6A) and bush bean (Fig. 2.7.6B) and several other species (Table 2.7.8). O$_3$ exposure had a strongly inhibitory effect on $g_s$, a commonly observed response that represents an active plant defense mechanism against oxidant injury (Massman et al., 2000). The observed values of $g_s$ are lower than commonly observed with these species under higher light field conditions.

The daily mean $g_s$ declined linearly with O$_3$ exposure in sorghum and bush bean (Fig. 2.7.6C, and D). The decline was proportional at the intermediate O$_3$ exposure in these species. In soybean and cotton (Table 2.7.8), exposure to 102 nL L$^{-1}$ induced a somewhat greater than proportional reduction in $g_s$, relative to 204 nL L$^{-1}$ O$_3$, resulting in a somewhat concave response.
With simultaneous measurement of O₃ concentration, we calculated flux of O₃ directly (Section 2.7.1; Grantz et al., 2014). Ozone-induced reduction in stomatal conductance reduced the relative increase in O₃ dose compared with the increase in concentration, at intermediate and high exposures relative to the low [O₃]. Averaged over all species, the increase in ozone concentration was 20.4- and 40.8-fold, respectively, whereas the increase in dose was only 14.1- and 18.8-fold (not shown). No relationship between foliar ClO₄⁻ and O₃ concentration was found (Fig. 2.7.5) and no relationship was found by use of O₃ dose, in any species (not shown) nor in the pooled data (Fig. 2.7.7A), despite a wide range of dosage achieved. A similar absence of relationships, both between foliar ClO₄⁻ and O₃ concentration and between ClO₄⁻ and O₃ dose, was observed at lower O₃ concentrations in both young and old leaf cohorts in the previous study (Section 2.7.1). The flux metric did not contribute to identification of meaningful relationships in either case.

Normalization of the data within each species by its median value (Fig. 2.7.7B) did not strengthen the statistical relationship, also as observed in the earlier study.
Table 2.7.8. Effect of exposure to ozone on stomatal conductance in contrasting species. Average of midday values (12:00 – 14:00).

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>$g_s$ at 4 nL L$^{-1}$ (mol m$^{-2}$ s$^{-1}$)</th>
<th>S.E.</th>
<th>% $g_s$ reduction relative to $g_s$ at 4 nL L$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100 nL L$^{-1}$ 204 nL L$^{-1}$</td>
</tr>
<tr>
<td>Soybean</td>
<td>18</td>
<td>0.14 b ± 0.020</td>
<td></td>
<td>27% 29%</td>
</tr>
<tr>
<td>Bush bean</td>
<td>18</td>
<td>0.13 b ± 0.027</td>
<td></td>
<td>39% * 63% *</td>
</tr>
<tr>
<td>Maize</td>
<td>18</td>
<td>0.055 c ± 0.0084</td>
<td></td>
<td>16% 73% *</td>
</tr>
<tr>
<td>Sorghum</td>
<td>20</td>
<td>0.082 bc ± 0.0093</td>
<td></td>
<td>18% 33% *</td>
</tr>
<tr>
<td>Pima Cotton</td>
<td>12</td>
<td>0.29 a ± 0.032</td>
<td></td>
<td>79% * 83% *</td>
</tr>
</tbody>
</table>

(*) significant at P < .05.
Figure 2.7.4. Significant relationship between perchlorate concentration (µg (kg dry wt)$^{-1}$) of potting medium exposed in the CSTRs and O$_3$ exposure (nL L$^{-1}$; 12 hr mean;  $r^2 = 0.99; P = 0.0714$). The data for unused medium and medium exposed to 4 and 114 nL L$^{-1}$ O$_3$ are from Grantz et al. (2014). Means followed by different letters are significantly different (P < 0.05).
Figure 2.7.5. No relationship between perchlorate concentration (µg (kg dry wt)^{-1}) of young leaves and O_3 exposure.
Figure 2.7.6. Effect of O₃ exposure on stomatal conductance ($g_s$). (A,B) Diel course of $g_s$ in (A) sorghum and (B) bush bean at low (open symbols) and high (closed symbols) O₃ exposure. (C,D) Relationship between $g_s$ of (C) sorghum and (D) bush bean (average of midday values, 12:00 – 14:00), and ozone exposure. Means followed by different letters are significantly different ($P < 0.05$).
Figure 2.7.7. No relationship between (A) perchlorate concentration of young leaves (µg (kg dry wt)^{-1}) and O₃ dose; or (B) perchlorate concentration (unitless) normalized by the median concentration over all O₃ within each species, and O₃ dose.
2.7.2.4 Conclusions
The ubiquitous distribution of vegetation, rising concentrations of tropospheric O_3, and occasional bursts of very high O_3 associated with stratospheric intrusion, combined with evidence that O_3 facilitates conversion of Cl\(^-\) to ClO_4\(^-\) under laboratory and field conditions, suggested that O_3-exposed vegetation may contribute to the emission inventory for ClO_4\(^-\) in the environment. We show that contrasting crop species all accumulate ClO_4\(^-\) from the growth medium, but with different bioconcentration factors. We further show that exposure to very high O_3 up to 320 nL L\(^{-1}\), hourly maximum, daily for 9 weeks, provided no evidence that foliar contents of ClO_4\(^-\) were related to O_3 exposure. There was no evidence that this relationship was strengthened by use of the measured uptake of O_3 into the leaf interior (O_3 flux or dose). These results are fully consistent with earlier results at more moderate concentrations of O_3. We found a trend of increasing soil surface content of ClO_4\(^-\) with increasing O_3 in both studies. The consistency between studies and the environmental significance of this potential source of ClO_4\(^-\) suggest that this putative soil pathway deserves further investigation. As the concentrations of O_3 used in these studies are at or above those observed even during extreme events of tropopause folding and in polluted mega-cities in the developing world, we conclude that O_3-exposed vegetation is not likely to be a significant source of environmental ClO_4\(^-\).
2.8 Effect of Plant Uptake on Stable Isotope Composition of ClO₄⁻

2.8.1 Background

A process which could affect ClO₄⁻ isotopes is plant uptake; however, its impact on ClO₄⁻ isotopic compositions is not yet clear. It is possible that plants could change the ClO₄⁻ isotopic composition through a variety of mechanisms including: transport carrier in the root; diffusion limitations through the root; reduction of ClO₄⁻ by plant reductases, translocation within the plant, and non-specific exchange of O between ClO₄⁻ and H₂O or other compounds catalyzed by plant compounds.

Uptake of ClO₄⁻ in plants has been studied extensively in both soil and hydroponic exposure experiments (Section 2.7; Sanchez et al., 2005a,b; Van Aken and Schnoor, 2002; Urbansky et al., 2000; Smith et al., 2004; Seyfferth et al., 2007, 2008 a,b; Yu et al., 2004; Nzengung et al., 1999, 2003, 2004; Tan et al., 2004a,b, 2006; Suslara et al., 2000; Ha et al., 2011; Voogt et al., 2010; Marschner, 1995). Most studies indicate ClO₄⁻ accumulation is species and genotype-dependent and most of the ClO₄⁻ accumulation occurs in transpiring tissues with large surface areas (e.g. leaves) (Sanchez et al., 2005a,b; Smith et al., 2004; Seyfferth et al., 2007, 2008 a,b; Nzengung et al., 1999; Voogt et al., 2010). From positive relations between the mass of ClO₄⁻ entering the plant and the amount of water being transpired, it is inferred that uptake of ClO₄⁻ is at least partially through passive transport, but demonstrated competition for uptake between ClO₄⁻ and other anions like NO₃⁻ and HCO₃⁻ from soil or water indicates that transport is not purely a passive process and that transport proteins may also facilitate transfer of ClO₄⁻ into plant cells (Seyfferth et al., 2008 a,b.; Tan et al., 2006; Suslara et al., 2000; Voogt et al., 2010; Marschner, 1995; Farquhar et al., 1989). In contrast to NO₃⁻ and HCO₃⁻ other anions such as Cl⁻, SO₄²⁻, I⁻, IO₃⁻ are not reported to interfere with ClO₄⁻ uptake (Seyfferth et al., 2008; Voogt et al., 2010; Marschner, 1995). The influence of NO₃⁻ on ClO₄⁻ uptake indicates that a similar uptake mechanism exists for both anions. Plant transporters are selective for specific anions on the basis of physicochemical properties, thus similarities in the properties of ClO₄⁻ and NO₃⁻ may cause competition for a common carrier protein or metabolic pathway within the plant tissue (Seyfferth et al., 2008; Farquhar et al., 1989).

It is well known that physiological and physicochemical processes (fixation, assimilation, and diffusion) in plants can cause isotopic fractionation of carbon (¹³C/¹²C) and nitrogen (¹⁵N/¹⁴N); (Evans, 2001; Mariotti et al., 1982; Tcherkez et al., 2006; Karsh et al., 2012). By analogy, intracellular processing of ClO₄⁻ within plants could also affect ClO₄⁻ isotope compositions. Isotopic discrimination occurs when NO₃⁻ is reduced and assimilated in plant tissue by the nitrate reductase enzyme, causing an increase in both δ¹⁵N and δ¹⁸O of the residual NO₃⁻ (Andraski et al., 2014). Although processes by which ClO₄⁻ might be metabolized within plants are unknown, considering that ClO₄⁻ and NO₃⁻ might share the same uptake mechanism, it is possible that ClO₄⁻
may be subjected to reduction and isotopic fractionation in plants by action of a catalyst similar
to nitrate reductase. At least one study (Van Aken et al., 2002) indicated ClO$_4^-$ reduction in
plants. Such processes in plants could affect the isotopic characteristics of ClO$_4^-$ returned to soils
and groundwaters if residual ClO$_4^-$ is released back into the environment, which appears likely
(Van Aken et al., 2002; Nzengung et al., 2004; Susalara et al., 2000; Laursen et al., 2013).
Improved understanding of such processes could be useful for interpreting origins of
environmental ClO$_4^-$ and NO$_3^-$ . The relative abundance and isotopic composition of ClO$_4^-$ and
NO$_3^-$ in plants could also provide evidence about fertilizer sources used to grow commercial
plant products (e.g. Mihailova et al., 2014; Hatzinger et al., 2011).

In this task, controlled experiments were conducted to develop procedures for analyzing the
isotopic composition of ClO$_4^-$ extracted from plants and to determine whether isotopic exchange
or fractionation (by transport or degradation) might alter the isotopic composition of ClO$_4^-$
accumulating in plants. This was accomplished by comparing the stable isotopic compositions
of ClO$_4^-$ accumulated in hydroponically grown snap bean plants (Phaseolus vulgaris L.) with
those of the starting reference materials and growth solutions. Results were interpreted in part by
contrasting the isotopic fractionation of ClO$_4^-$ and NO$_3^-$ in the same experiments. This is
important because isotopic fractionation effects of enzymatic reactions may not fully be
expressed in residual reactants (i.e. in heterogeneous systems where reactions are transport-
limited). Therefore, a lack of observable isotope effects in extractable ClO$_4^-$ might not be a
reliable indicator of ClO$_4^-$ stability unless extractable NO$_3^-$ exhibited substantial fractionation
effects. We also evaluated ClO$_4^-$ isotopic composition in plants and source water for field-grown
snap beans to validate the applicability of our methods in a more realistic setting. In order to
demonstrate how our methods can be used to identify sources of ClO$_4^-$ in the food chain, we
analyzed commercially grown spinach and snap bean plants exposed only to background
concentrations of ClO$_4^-$. 

2.8.2 Materials and Methods

2.8.2.1 Hydroponic Experimental Design

Hydroponic greenhouse studies were conducted at the Texas Tech University Greenhouse
Complex in Lubbock, TX. Snap beans (Phaseolus vulgaris L.) were grown hydroponically in
nutrient solutions containing either normal synthetic reagent ClO$_4^-$ or isotopically labeled ClO$_4^-$.
Plants were harvested at maturity, when bean pods were full grown and flowering ceased. Plants
were divided into roots, stems, leaves, and bean pods and each compartment was evaluated for
ClO$_4^-$, NO$_3^-$, Cl$^-$ uptake. Isotopic analyses were performed on ClO$_4^-$ and NO$_3^-$ in bean leaves and
growth solutions. Details are described below.
Two hydroponic experiments were conducted with multiple treatments in each experiment (Table 2.8.1). Treatments generally consisted of plants grown in nutrient solutions containing ClO$_4^-$ (0.01, 2, and 10 mg/L) along with NO$_3^-$ and other ions. Two control treatments were also evaluated. One control contained no plants and 1 mg/L ClO$_4^-$ and the other contained plants and had no ClO$_4^-$. The ClO$_4^-$ for the 0.01, 1, and 10 mg/L exposures was supplied from a 98% sodium perchlorate (NaClO$_4$) lab reagent (ACROS Organics) while the ClO$_4^-$ used in the 2 mg/L exposure was supplied from an isotopically labeled KClO$_4$ reagent (USGS38) distributed by the USGS (Reston, VA). All treatments were evaluated to determine the amount of ClO$_4^-$ and NO$_3^-$ lost from solution over time and the amount of ClO$_4^-$ and NO$_3^-$ accumulated in plant tissue, while a subset of treatments (1 mg/L no plants control, 2 mg/L ClO$_4^-$, and 10 mg/L ClO$_4^-$) were evaluated for stable isotopes.
Table 2.8.1 Summary of initial experimental conditions for ClO$_4^-$ treatments in experiment 1 and 2. Experiment 1 was conducted during the months of October and December while experiment 2 was conducted during April and May.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1 ClO$_4^-$ Treatments (mg/L)</th>
<th>Experiment 2 ClO$_4^-$ Treatments (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cl$^-$ (mg/L)</td>
<td>1.2 (0)</td>
<td>1.0 (0)</td>
</tr>
<tr>
<td>NO$_3^-$-N (mg/L)</td>
<td>274 (0)</td>
<td>268 (0)</td>
</tr>
<tr>
<td>PO$_4^{3-}$ (mg/L)</td>
<td>48 (0)</td>
<td>46 (0)</td>
</tr>
<tr>
<td>SO$_4^{2-}$ (mg/L)</td>
<td>117 (0)</td>
<td>110 (0)</td>
</tr>
<tr>
<td># tubs (replicates)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Plants per tub</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Anion concentrations reported are the means of the replicates for each ClO$_4^-$ treatment. Standard errors of the means are indicated in parenthesis (e.g. Mean (±SD)).

Snap bean (*Phaseolus vulgaris* L.) seeds were sown in small rockwool cubes presoaked in reverse osmosis (RO) water. The seeds were watered daily with RO water until full germination (presence of 4-5 leaves, ~3 weeks) was achieved. During the germination period, the small rockwool cubes were transferred to larger rockwool cubes. Polypropylene/polyethylene opaque tubs measuring 52.7cm × 36.87cm × 33.3cm and pre-rinsed with RO water three times were filled with 32 L and 34L (for experiment 1 and experiment 2, respectively) of a standard nutrient solution that was made by the methods of a previous study (Marschner, 1995) with minor modifications (Table 2.8.1). Prior to transfer of the plants to the growth tubs, the pH of the nutrient solution was adjusted to 5.5-6.1 by addition of HNO$_3$. The rockwool cubes containing plants were then positioned on the lids of the tubs, which were then secured to the tubs with the plant roots hanging into the nutrient solution and plants exposed to air. Rockwool cubes were covered with aluminum foil to prevent algae growth. Commercial aquarium pumps supplied air to maintain bulk oxic conditions in solution for the duration of the experiments.

Once germination reached the 4-5 leaf stage (~15-18 days), plants were exposed to ClO$_4^-$ by addition of a concentrated solution in selected tubs. The water level in each tub was maintained by adding RO water weekly. Growth solution samples from each tub were collected every two to three days and refrigerated immediately until they could be analyzed. Electrical conductivity and pH were also measured two to three times weekly. To maintain pH between 5.5 and 6.1, 3M KOH was added as needed. After some weeks, insects became apparent and leaves were treated

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using Safer Soap (AgroCrop Sciences). Eventually, insects became so problematic the plants were sprayed with Orthonex (an organophosphate insecticide).

Snap bean plants were harvested 62-65 days after germination, corresponding to 38 days of ClO₄⁻ exposure. The bean pods, leaves, stems, and roots from each treatment were separated, weighed, and then placed in labeled plastic bags. The plastic bags were placed in a freezer until processed. The growth solution and rockwool cubes in which the plants were grown were also weighed and frozen until processed.

### 2.8.2.2 Sampling and Processing of Hydroponic Solutions

The plant solute extraction method of Ellington and Evans, (2000) was used in these experiments with minor modifications. Leaves, bean pods, stems, and roots were separated into approximately 20, 5, 6, and 10 g fresh weight (FW) portions, respectively. Each portion of leaves, bean pods, stems, and roots were placed in containers (vials) with 200, 25, 25, and 20 mL of distilled deionized (DDI) water, respectively. Water/plant tissue mixtures were boiled for 30-60 minutes in a precision boiler set at 99°C. The boiled extracts were centrifuged and the supernatant was decanted into containers (vials) with activated alumina (Al₂O₃) and placed in the refrigerator overnight. Each leaf aqueous extract was then vacuum filtered using GF/F 47 mm Whatman™ filters. The bean pod, stem, and root extracts were filtered using syringe Whatman™ filters. From each tissue extraction solution, a small volume was separated and cleaned by passing the aqueous extract through a (0.2 µm) syringe filter and a Dionex RP cartridge. Cleaned samples from each treatment were analyzed for ClO₄⁻, Cl⁻, NO₃⁻-N, SO₄²⁻, and PO₄³⁻ and a small volume was set aside and preserved for NO₃⁻ isotopic analysis by raising the pH to 11-12 using 50% w/w NaOH. The remaining filtered supernatants for each tub in each treatment were pooled together to make one extraction solution per treatment for ClO₄⁻ isotopic analysis. The pooled leaf extracts for each treatment as well as the remaining nutrient solutions, excluding the 0.01 mg/L and no ClO₄⁻ control plant extraction and growth solutions, were pumped through Purolite A-530E resin columns to capture the ClO₄⁻ (Gu et al., 2011). Columns were preserved with 0.1 N HCl and stored at 4°C until processing.

### 2.8.2.3 Snap Bean Field Studies

Ozone-sensitive (S156) and tolerant (R123) snap bean genotypes described previously (Burkey et al., 2005) were grown in field plots during the summers of 2009 – 2011 at two locations to provide bulk leaf tissue for extraction and isotopic analysis of ClO₄⁻. The first site was located at the USDA-ARS Air Quality field site 5 km south of Raleigh, NC (Lat: 35°43'59" N; Long: 78°41'2" W) and the second site was located at the Long Island Horticultural Research & Extension Center, Riverhead, NY (Lat: 40°57'36" N; Long: 72°43'12" W). For each location in each year, three separate plots consisting of 500 linear feet of row were established as three experimental blocks with each block consisting of one S156 plot and one R123 plot (randomized
complete block design). Fields were cultivated before planting and a ‘10-10-10 Extra’ fertilizer blend (Carolina Eastern-Vail, Niverville, NY) was applied at a rate of 500 lbs/acre by broadcast and incorporated into the soil prior to planting. Approximately 1500 seeds were sown per plot in late May or early June each year at both sites. Plots were provided water equivalent to at least 1 inch of rain per week by either natural rainfall or supplemental drip irrigation from wells located at each site. In preliminary tests, well water at the NC site was found to contain a relatively high concentration ClO$_4^-$ (~ 5 µg/L). In this case, a Purolite® A-530E resin filter was used to reduce ClO$_4^-$ levels. Irrigation water at the NY site was found to contain ~0.3 ppb ClO$_4^-$, and filtering was not deemed necessary. At 45-55 days after planting at the developmental stage where vegetative biomass was near maximum, leaf tissue was manually harvested from each plot, placed in mesh bags, and dried at 60 °C. Dried leaf tissue was stored in large plastic bags to exclude moisture and prevent contamination and then shipped to Texas Tech University for ClO$_4^-$ extraction and purification.

Field grown snap bean leaves were extracted two times for 48 hours in large 55-gallon barrels using reverse osmosis water to which the pH was reduced to 2 using HCl. Leaves were removed from solution and the solution was pumped through a 0.45 µm filter and Purolite® A-530E resin columns. The resin columns were stored at 4°C until processed for ClO$_4^-$ isotopes.

2.8.2.4 Sampling and Processing of Spinach

Thirty-eight (38) kg of fresh spinach leaves in pre-packaged plastic bags was purchased from a supermarket in Lubbock, Texas. Spinach sold by the supplier was conventionally grown by the NewStar Fresh Foods Limited Liability Company in the Salinas Valley, California region from April through October and the Yuma, Arizona region from November through March. The spinach was purchased in early November and thus it is not known which location the spinach was from. The spinach was frozen to rupture the plant cell walls and facilitate ion extraction.

Solutions were extracted using a spinach to water ratio of 0.28 by weight (19 kg / 84 kg). The leaves were mixed thoroughly in the water and were allowed to extract overnight. Leaves were then removed from the solutions and the extraction solution was pumped through a 0.45 µm filter and Purolite A-530E resin column. The extraction column was preserved with HCl at a pH ≤ 2.

2.8.2.5 Sample Analyses

Concentrations of ClO$_4^-$ and major anions (Cl$^-$, NO$_3^-$, SO$_4^{2-}$, PO$_4^{3-}$) in hydroponic solutions and plant extracts were measured at Texas Tech University using sequential ion chromatography-mass spectroscopy-mass spectroscopy (IC-MS/MS) with a reporting limit of 0.05 µg/L (ClO$_4^-$) and ion chromatography (EPA Method 300.0) with a reporting limit of 0.5 mg/L (other anions) as previously described in this report. The ClO$_4^-$ in the resin columns was extracted and purified.
at Oak Ridge National Laboratory using procedures described previously (Hatzinger et al., 2011; Gu et al., 2011). Purified ClO₄⁻ was shipped to the USGS in Reston Virginia for analysis of δ¹⁸O, δ¹⁷O, and Δ¹⁷O of O₂ produced by decomposition of ClO₄⁻ and the Cl⁻ residue from the decomposed ClO₄⁻ was analyzed for δ³⁷Cl at the University of Illinois at Chicago (Hatzinger et al., 2011). The NO₃⁻ stable isotope ratios (δ¹⁸O, δ¹⁵N) of hydroponic solutions and plant extracts were also analyzed at the USGS in Reston, Virginia (Sigman et al., 2001; Casciotti et al., 2002; Böhlke et al., 2003, 2007). Isotopic reference materials and calibration values for ClO₄⁻ and NO₃⁻ were from Böhlke et al. (2009) and Böhlke et al. (2003), respectively. Selected samples were analyzed for NO₂⁻ concentration and isotopic composition, as NO₂⁻ can interfere with NO₃⁻ isotopic analysis (Böhlke et al., 2007).

2.8.3 Results

2.8.3.1 Hydroponic Study

Changes in the Growth Solutions

Substantial water loss occurred in both hydroponic experiments (Figure 2.8.1). This loss was attributed mainly to transpiration, as the cumulative volume of growth solution lost in treatments with no plants was less than 10% of the initial volume compared to a cumulative loss greater than 96% of the initial volume in treatments with plants (Figure 2.8.1). Within each experiment the volume of solution transpired by plant treatments was similar regardless of the presence or absence of ClO₄⁻, but the volume transpired in experiment 2 was approximately twice that of experiment 1 (Figure 2.8.1). Experiment 1 was conducted during the months of October and December and experiment 2 during the months of April and May when temperatures were higher, possibly contributing to higher transpiration in experiment 2. Individual tubs contained more plants in experiment 1, but plant mass was 2-4 fold higher in experiment 2, possibly contributing to more overall transpiration in experiment 2.
Figure 2.8.1. Cumulative volume of growth solution uptaken by pooled replicate treatments in both experiments.
Cumulative volume refers to the overall volume of nutrient solution uptaken by the snap bean plants in each treatment from time of exposure to nutrient solution to time of harvest. Values plotted are average values of the replicates in each treatment. No replicate is available for the control treatments or for the 0.01 mg/L ClO₄⁻ treatment.
As DDI water was added to replace transpired water, ClO$_4^-$ concentrations in the hydroponic tubs generally should have not changed as a result of dilution, but they were affected by selective uptake into plants. Therefore, our results are reported on a mass basis hereafter. As in previous studies (Van Aken et al., 2002; Seyfferth et al., 2007; Tan et al., 2006), ClO$_4^-$ mass in hydroponic solutions of treatments with plants decreased gradually over time in a non-linear fashion (Figure 2.8.2a and 2.8.2d). On a cumulative mass loss basis, our results indicate an overall loss of 60-80% of the initial ClO$_4^-$ from solution (Figure 2.8.2a and 2.8.2d). The fractional mass loss of ClO$_4^-$ from solution was independent of initial ClO$_4^-$ concentration, but there was a larger overall decrease in ClO$_4^-$ solution mass in experiment 2, which had higher transpiration loss of water (Figure 2.8.2d and Table 2.8.2). There was an unexplained decrease (~20%) in ClO$_4^-$ solution mass in treatments with no plants in experiment 1, but not experiment 2. Linear correlations that were independent of initial ClO$_4^-$ treatment concentration ($r^2 = 0.82$ and 0.95 for 2 mg/L and 10 mg/L ClO$_4^-$, respectively, $P < 0.05$) were observed between the amount of solution transpired and the fraction of ClO$_4^-$ solution mass loss in experiment 2 (Figure 2.8.3a). Insufficient time points were available to evaluate such correlations in the first experiments. Our results support previous work (Seyfferth et al., 2007; Tan et al., 2004) that suggests ClO$_4^-$ mass lost from solution is directly proportional to the volume of transpired water, but less than the predicted amount based on water uptake and the concentration in solution.
Figure 2.8.2. Fraction of ClO₄⁻, Cl⁻, and NO₃⁻ mass remaining in nutrient solutions at the time of harvest. Values reported are the average of pooled replicates in each treatment. Experiment 1 data is represented in graph panels (a), (b), and (c) while experiment 2 data is represented in panels (d), (e), and (f). No replicate is available for the control treatments or for the 0.01 mg/L ClO₄⁻ treatment.
Like ClO₄⁻, the NO₃⁻ concentrations in solutions of treatments with plants decreased gradually over time in a non-linear fashion and there was smaller NO₃⁻ loss (< 20% of total NO₃⁻) in the no plant treatments (Figure 2.8.2c and 2.8.2f and Table 2.8.2). The NO₃⁻ fractional mass loss was linearly correlated (P < 0.05) with the amount of solution transpired regardless of initial ClO₄⁻ treatment concentration ($r^2 = 0.76, 0.83, 0.86$ for 0, 0.01, and 10 mg/L ClO₄⁻, respectively) (Figure 2.8.3c). Both NO₃⁻ and ClO₄⁻ had similar uptake rates as % mass/L transpired (-0.8683 ± 0.15 to -0.9912 ± 0.21) and (-0.97 ± 0.08 to -1.02 ± 0.17), respectively, supporting a similar uptake mechanism, as also suggested previously by other studies (Seyfferth et al., 2008; Marschner, 1995).

In contrast to ClO₄⁻ and NO₃⁻, Cl⁻ mass in solution remained relatively constant (~ 80-120%) throughout the experiment regardless of initial ClO₄⁻ treatment concentration and presence or absence of plants (Figure 2.8.2e). There was no correlation between mass of Cl⁻ removed from solution and the amount of solution transpired ($r^2 = 0.0001-0.2255$, P > 0.05 for all treatments combined) (Figure 2.8.3b). Although Cl⁻ clearly was accumulated in plants, the amount taken up was small compared to the amount in solution (high – Cl⁻ in experiment 2) or small compared to analytical uncertainty (low – Cl⁻ in experiment 1).
Table 2.8.2. Average ClO₄⁻, NO₃⁻, Cl⁻ and total-N fractional mass distribution in growth solution and snap bean plant tissue.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1: ClO₄⁻ Treatments (mg/L)</th>
<th>Experiment 2: ClO₄⁻ Treatments (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Final Solution</td>
<td>N/A</td>
<td>62.5(0.0)</td>
</tr>
<tr>
<td>Leaves</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Bean Pods</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Stems</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Roots</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Rockwool</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Unaccounted</td>
<td>N/A</td>
<td>37.5(0.0)</td>
</tr>
</tbody>
</table>

% ClO₄⁻ mass of initial total ClO₄⁻ mass (SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1: NO₃⁻ Treatments (mg/L)</th>
<th>Experiment 2: NO₃⁻ Treatments (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Final Solution</td>
<td>57.1(0.0)</td>
<td>84.3(0.0)</td>
</tr>
<tr>
<td>Leaves</td>
<td>0.45(0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Bean Pods</td>
<td>0.45(0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Stems</td>
<td>0.52(0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Roots</td>
<td>0.31(0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Rockwool</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>Unaccounted</td>
<td>41.2(0.0)</td>
<td>15.7(0.0)</td>
</tr>
</tbody>
</table>

% NO₃⁻ mass of initial total NO₃⁻ mass (SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1: CF mass (mg)</th>
<th>Experiment 2: CF mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Final Solution</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Leaves</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Bean Pods</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Stems</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Roots</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Rockwool</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Unaccounted</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

% CF mass of initial total CF mass (SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1: total-N mass (mg)</th>
<th>Experiment 2: total-N mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Final Solution</td>
<td>N/A</td>
<td>57.1(0.0)</td>
</tr>
<tr>
<td>Leaves</td>
<td>12.8(0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Bean Pods</td>
<td>14.3(0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Stems</td>
<td>3.8(0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Roots</td>
<td>2.7(0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Rockwool</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>Unaccounted</td>
<td>9.4(0.0)</td>
<td>15.7(0.0)</td>
</tr>
</tbody>
</table>

% total-N mass of initial total-N mass (SD)

NM = Not Measured
N/A = Not Applicable
(SD) = Standard deviation
Figure 2.8.3. Relationship between fraction of total (a) ClO$_4^-$, (b) Cl$^-$, and (c) NO$_3^-$ mass lost from solution and cumulative volume of solution transpired over the course of experiment 2. The grayish yellow regression lines correspond to the 0.01 mg/L ClO$_4^-$ treatment, the dark blue regression lines correspond to the 10 mg/L ClO$_4^-$ treatment, and the aqua blue regression lines correspond to the no ClO$_4^-$ with plants treatment. Not enough data was collected for an analysis of experiment 1. No replicate is available for the 0.01 mg/L treatment.
Anion Distributions and Bioconcentration Factors

Whole plants were sectioned and solutes were extracted from plant tissue to determine how much $\text{ClO}_4^-$ from solution had accumulated in the plant. Plant uptake of $\text{ClO}_4^-$ was proportional to exposure concentration in both experiments, but more $\text{ClO}_4^-$ mass was accumulated in experiment 2 for the same exposure concentrations, likely because of more total loss of H$_2$O by transpiration in experiment 2. To facilitate comparisons, leaf bioconcentration factors (BCF) were calculated using Equation 1.

\[
\text{BCF} = \frac{\text{Ion mass per plant fresh weight at harvest (mg/kg)}}{\text{Initial ion concentration in solution (mg/L)}}
\] (1)

Combined results of both experiments yielded $\text{ClO}_4^-$ BCF values of 66 ± 0, 64 ± 3, and 63 ± 11 L/kg fresh weight (FW) for the 0.01, 2, and 10 mg/L $\text{ClO}_4^-$ treatments, respectively. Our BCF values were higher than those reported for lettuce (2-25 L/kg; Seyfferth et al., 2007) but within the range of those reported for spinach (17-102 L/kg; Voogt et al., 2010). The distribution of $\text{ClO}_4^-$ between the different plant parts was independent of $\text{ClO}_4^-$ exposure concentration in both experiments (Table 2.8.2). Of the $\text{ClO}_4^-$ found in the plant, the majority was located in leaves (77-90%), a finding consistent with past studies (Jackson et al., 2005; Seyfferth et al., 2007, 2008; Nzengung et al., 2003, 2004; Voogt et al., 2010). The next largest accumulation of $\text{ClO}_4^-$ was detected in bean pods (4-21%), followed by stems (1.2-2.8%), and then roots (1-2%). A mass balance was performed to determine the fate of $\text{ClO}_4^-$ lost from solution. Approximately 27.3 ± 4.4 to 42.0 ± 7.3% and 14.3 ± 0.0 to 19.8 ± 3.4 % of the initial $\text{ClO}_4^-$ mass was unaccounted for in experiment 1 and experiment 2, respectively (Table 2.8.2). The solute extraction procedure was applied to the rockwool cubes in which snap bean seeds were sown at the end of experiment 1 to determine if they were a possible sink for missing $\text{ClO}_4^-$, but the amount of $\text{ClO}_4^-$ recovered was negligible compared to the amount unrecovered (Table 2.8.2).

BCF values were also calculated for $\text{NO}_3^-$ in both experiments (1.56 ± 0.76, 0.49 ± 0, 1.38 ± 0.11, and 1.40 ± 0.63 L/kg for 0, 0.01, 2, and 10 mg/L $\text{ClO}_4^-$, respectively), but these values are not meaningful as most of the $\text{NO}_3^-$ was converted within plants to organic N. Only a small amount of $\text{NO}_3^-$ was detected in plant tissues, with the accumulation being higher in the experiment 2, which had higher transpiration (Table 2.8.2). Fractional mass of plant $\text{NO}_3^-$ was relatively similar among different plant tissues. Much of the $\text{NO}_3^-$ lost from solution (~ 15.7 ± 0 to 43.5 ± 6.4% and 8.0 ± to 72.3 ± 6.9% in experiment 1 and experiment 2, respectively) was recoverable as N in plant tissues, as indicated by the near-quantitative recovery of the initial total-N (Table 2.8.2). Experiment 1 BCF average values for total-N were 158, 181, and 159 for 0, 2, and 10 mg/L $\text{ClO}_4^-$ treatments, respectively.

The BCF values for $\text{Cl}^-$ (1.85, 8.76, and 3.74 L/kg for the 0, 0.01, and 10 mg/L $\text{ClO}_4^-$ treatments in the second experiment, respectively) were much lower than those for $\text{ClO}_4^-$ indicating that
plants selectively uptake ClO$_4^-$ as opposed to Cl$^-$. Plants did not accumulate much Cl$^-$, but of the Cl$^-$ in the plant, Cl$^-$ percent mass was distributed similarly between leaves and stems (24-43%) and between bean pods and roots (10-24%). The fraction of Cl$^-$ mass recovered in the snap bean plant and the Cl$^-$ mass not accounted for were comparable (~9-15%) (Table 2.8.2).

**Perchlorate Isotopes**

The role of plant uptake on ClO$_4^-$ isotopic composition was determined by evaluating the isotopic composition of solution and plant leaf ClO$_4^-$. To minimize ambiguity in the results, two sources of ClO$_4^-$ were used in the experiments: a normal synthetic NaClO$_4$ lab reagent and a KClO$_4$ salt that was depleted in $^{37}$Cl and enriched in $^{18}$O and $^{17}$O (USGS38, with large positive $\Delta^{17}$O). With few exceptions, leaf and solution ClO$_4^-$ isotopic composition ($\delta^{37}$Cl, $\delta^{18}$O, $\Delta^{17}$O) were not consistently different from each other in both experiments using both ClO$_4^-$ sources ($n = 4$ pairs) (Figure 2.8.4). For most experiments using isotopically anomalous ClO$_4^-$ (Figure 2.8.4a and 2.8.4b), small differences between final growth solutions and leaf extracts were not systematically in one direction or the other, and likely represent small variations or impurities or isotope effects of sample preparation. One of the 2 mg/L ClO$_4^-$ replicate treatments in experiment 1 had anomalously high $\delta^{18}$O and relatively high $\delta^{37}$Cl in leaf tissue, but not difference in $\Delta^{17}$O (Figure 2.8.4a and 2.8.4b). That sample may have been isotopically fractionated slightly, but it is not known if the anomalous result was caused by plant processes or subsequent handling of the organic-rich extract. The NO$_3^-$ extracted from that sample was not anomalous. For experiments with normal reagent ClO$_4^-$ (Figure 2.8.4c and 2.8.4d), small apparent trends could be consistent with fractionation effects in growth solutions or plants, but overall variations were $\leq 1\%$. Given typical uncertainties of approximately $\pm 0.5$ to $\pm 1.0\%$ due to combined effects of ClO$_4^-$ extraction, purification, and isotopic analysis, these data indicate isotopic fractionation or exchange attributable to plant ClO$_4^-$ uptake, plant ClO$_4^-$ transformation, microbial ClO$_4^-$ transformation, or isotopic exchange between ClO$_4^-$ and other constituents during the experiments was generally insignificant.
Figure 2.8.4. Stable ClO$_4^-$ isotopic composition of nutrient solutions and leaf extracts of replicate treatments in experiment 1 and 2. Experiment 1 is represented by the red triangle, green circle, and light blue cross symbols, while experiment 2 is represented by the dark blue square and pink diamond symbols. $\delta^{37}$Cl, $\delta^{18}$O (O$_2$-DI), and $\Delta^{17}$O values are referenced to 0 for SMOC, VSMOW, and VSMOW, respectively.
Nitrate Isotopes

Hydroponic solution NO₃⁻ δ¹⁵N values appear to have increased slightly (< 2 %) over time in both experiments for all treatments with plants but no increase was observed in control treatments without plants (Figure 2.8.5). In experiment 1, δ¹⁵N increases were small but fairly constant throughout the growth period, whereas in experiment 2 the increases did not occur until mid-way through the growth period. In the two treatments with the largest δ¹⁵N increases, δ¹⁸O also increased significantly, whereas δ¹⁸O variations in other treatments were within normal analytical uncertainties. Apparent isotope fractionation factors (ε) derived from residual NO₃⁻ in solution were of the order of -0.6 to -1.5 ‰ for ε(15/14) and -0.4 to -3.1 ‰ for ε(18/16) (Figure 2.8.6). Correlated increases in δ¹⁵N and δ¹⁸O in late stages of two treatments could indicate minor fractionation by plant uptake or NO₃⁻ reduction, but the apparent effects in solution were much smaller than those attributable to assimilation within the plants (Figure 2.8.5). Apparent ε¹⁵N/ε¹⁸O values for NO₃⁻ in solution of around 0.5-0.6 (Figure 2.8.6f) were lower than most reported values for NO₃⁻ reduction (ε¹⁵N/ε¹⁸O ≈ 1) and possibly more like those reported for NO₃⁻ transport from solution into plants (Needoba et al., 2003, 2004; Granger et al., 2010) though overall uncertainties are large.
Figure 2.8.5. Stable isotopic composition of NO$_3^-$ in nutrient solutions as a function of time for experiment 1 (a, b) and experiment 2 (d, e). Panels (c) and (f) show the corresponding relation between $\delta^{15}$N and $\delta^{18}$O. Initial NO$_3^-$ isotopic compositions differed between the experiments. Only a subset of solution samples was analyzed in experiment 2.
Figure 2.8.6 Rayleigh plots of the change in the $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$ in nutrient solutions of experiment 1 (a, b, c) and experiment 2 (d, e, f). The colors of the regression lines and text are the same as those of the symbols for the treatments they represent (e.g. red = 2 mg/L ClO$_4^-$).
\(\delta^{15}N\) and \(\delta^{18}O\) values of NO\(_3^-\) extracted from leaves were higher than those in the hydroponic solutions (by about 10-20‰) (Figure 2.8.7). In both experiments there were linear (\(P < 0.001\)) correlations between \(\delta^{15}N\) and \(\delta^{18}O\) values of leaves and growth solutions with ratios (\(\Delta^{15}N/\Delta^{18}O\)) of 1.07 (experiment 1) and 1.04 (experiment 2), consistent with reported effects of NO\(_3^-\) reduction during assimilation by plants (Granger et al., 2004; Trull et al., 2008, DiFiore et al., 2009). This is supported by \(\delta^{15}N\) measurements of plants from experiment 1. Total N-\(\delta^{15}N\) varied by around 3.4 ‰ in different plant compartments (i.e. roots, stems, leaves, and bean pods). Highest \(\delta^{15}N\) values were in stems (\(\pm 34 \pm 3\) ‰) and lowest values were in roots (\(\pm 29 \pm 4\) ‰). The weighted composite mean \(\delta^{15}N\) value for whole plants in experiment 1 was +31± 4 ‰. A mass balance on total N in Experiment 1 indicates that at least 84 – 91% of the total initial mass of total-N is accounted for in residual growth solutions and whole plant tissue (Table 2.8.2). In experiment 1, \(\delta^{15}N\) values were ~ + 54 ‰ for solution NO\(_3^-\), ~ + 31 ‰ for final plant tissue, and ~+72‰ for final residual NO\(_3^-\) extracted from leaves. These results are quantitatively consistent with isotope effects dominated by internal NO\(_3^-\) reduction during assimilation. Plant total-N isotope data are not available for experiment 2, but isotopic contrasts between solution NO\(_3^-\) and leaf NO\(_3^-\) were similar to those in experiment 1, even though the NO\(_3^-\) reagents used in the experiments had different initial isotopic compositions (Figure 2.8.7).
Figure 2.8.7. Stable isotopic composition of NO$_3^-$ in final growth solutions (solid symbols) and leaf extracts (open symbols) of pooled replicate treatments in experiments 1 and 2. Plant total-N $\delta^{15}$N was only measured in experiment 1.
2.8.3.2 Snap Bean Field Study Results

ClO$_4^-$ isotopic values of field-grown snap beans from Raleigh, NC varied between years. The isotopic composition of plants grown in 2009 was intermediate and located on a two component mixing line between the isotopic composition of ClO$_4^-$ in groundwater used for irrigation and the isotopic composition of indigenous ClO$_4^-$ from groundwater in the US (Jackson et al., 2010) (Figure 2.8.8). In 2010, the isotopic composition of the snap beans was indistinguishable, given cumulative error terms (< ± 2‰), to the isotopic composition of ClO$_4^-$ in the groundwater. The isotopic composition of ClO$_4^-$ in the groundwater was very similar to the composition of Atacama ClO$_4^-$, consistent with the elevated ClO$_4^-$ concentrations and documented historical use of Chilean fertilizers. The difference in isotopic composition between years is qualitatively consistent with the variation in the mass of applied ClO$_4^-$ from each source (groundwater and atmospheric deposition) between years based on variations in rainfall and ClO$_4^-$ concentration in applied groundwater (Table 2.8.3).

Snap beans grown at Raleigh, NC received varying proportions of rainfall and groundwater in 2009 and 2010 (Table 2.8.3). Concentrations of ClO$_4^-$ in applied groundwater also varied between 2009 and 2010 (0.6 and 2.2 μg/L, respectively), presumably due to exhaustion of the ion exchange column installed to remove ClO$_4^-$ from applied groundwater (Table 2.8.3). The increase of applied ClO$_4^-$ from groundwater is reflected in the ~ 5X higher foliar ClO$_4^-$ concentrations in 2010 (X and Y, respectively). The similarity between the snap bean and groundwater isotopic composition in 2010 is consistent with the much larger applied ClO$_4^-$ mass from groundwater in 2010, (48 and 480 μg/m$^2$-yr (growing season only), in 2009 and 2010, respectively) (Table 2.8.3). The intermediate isotopic composition of foliar ClO$_4^-$ in 2009 is consistent with a larger contribution from indigenous ClO$_4^-$ due to the order of magnitude decrease in groundwater applied ClO$_4^-$ (i.e., containing Atacama ClO$_4^-$). Annual average ClO$_4^-$ deposition rate in the US is ~ 6.5 μg/m$^2$-yr with an unknown contribution from dry deposition.

The ClO$_4^-$ composition in snap beans from Long Island appears to reflect multiple sources, possibly dominated by indigenous ClO$_4^-$ and Atacama ClO$_4^-$ with a smaller contribution of manmade ClO$_4^-$ (Figure 2.8.8). It is not possible to determine if the foliar ClO$_4^-$ isotopic composition is consistent with the contributing sources as no data are available on the isotopic composition of the groundwater. Long Island groundwater has previously been shown to be a mix of indigenous and Atacama ClO$_4^-$ and given the very low concentrations of ClO$_4^-$ in the groundwater (~ 0.4 μg/L), the isotopic data are not unreasonable (Böhlke et al., 2009). Regardless, we show that for field-grown plants even with background levels of foliar ClO$_4^-$, it is possible to evaluate foliar isotopic composition.
Figure 2.8.8. Summary of isotope data for ClO$_4^-$ from Raleigh, NC and Long Island, NY field studies and commercial spinach displayed with previously published ClO$_4^-$ isotope data from synthetic and natural sources from the Atacama Desert in Chile, Death Valley (DV), California, and the Southern High Plains (SHP) and Middle Rio Grande Basin (MRGB) of Texas and New Mexico (Jackson et al., 2010; Böhlke et al., 2005; Bao and Gu, 2004; Hatzinger et al., 2011). Symbols with error bars for the 2010 Raleigh bean S156 (n = 3) and R123 (n = 2) plots indicate average values (± standard deviation). The mixing line (dark blue) was calculated assuming a two-member source (Raleigh groundwater and RGB/SHP) isotopic mass
2.8.3.3 Spinach Study Results

We analyzed commercially grown spinach as a demonstration of our extraction and analysis procedures and to evaluate sources of ClO$_4^-$ in food products. The spinach ClO$_4^-$ isotopic composition: $\delta^{37}$Cl = + 3.3 ‰, $\delta^{18}$O = + 3.7 ‰, $\Delta^{17}$O = + 2.6 ‰ was similar to isotopic compositions reported for indigenous natural ClO$_4^-$ in soil and groundwater in the southwestern U.S. and surface waters of the Great Lakes, and to inferred isotopic values for groundwater in parts of southern California (Figure 2.8.8) (This report, Sections 2.2 & 2.3; Jackson et al., 2010; Böhlke et al., 2005; Hatzinger et al., 2015; Sturchio et al., 2006; Sturchio et al., 2014; Poghosyan et al., 2014).

2.8.4 Discussion

A number of processes could contribute to the observed range of natural ClO$_4^-$ stable isotopic composition in soil and groundwater. Uptake of ClO$_4^-$ by ion transporters, plant-catalyzed reduction, or oxygen exchange could all potentially cause isotopic fractionation. Snap bean plants were found to selectively uptake ClO$_4^-$ relative to Cl$^-$, but not relative to NO$_3^-$, supporting a common ion transport mechanism for ClO$_4^-$ and NO$_3^-$. The similarity in uptake rates (-0.87 and -0.97 % mass/L) for ClO$_4^-$ and NO$_3^-$, the strong correlation of anion uptake with volume of transpired solution, and the lower than predicted ClO$_4^-$ mass accumulated in the plant (based on transpiration volume) are all consistent with findings from past studies (Seyfferth et al., 2008; Tan et al., 2006). This suggests that transport is through an ion carrier in roots that is specific to both NO$_3^-$ and ClO$_4^-$ but also dependent on transpiration rate (Seyfferth et al., 2008; Marschner, 1995).

No substantial differences were observed in the $\delta^{37}$Cl and $\delta^{18}$O values of ClO$_4^-$ between the growth solutions and leaf extracts during the hydroponic experiments. There were small, but inconsistent changes in stable isotopic composition of NO$_3^-$ in growth solutions. In experiment 1 a slight increase (~1‰) in $\delta^{15}$N-NO$_3^-$ but lack of measurable change in $\delta^{18}$O could be consistent with fractionation. The effects of NO$_3^-$ reduction or transport processes in the plant on $\delta^{18}$O may not be as apparent as for $\delta^{15}$N due to the difference in analytical uncertainty between the two (~ ± 1‰ versus ~ ± 0.5 ‰, respectively). In experiment 2, small increases in $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$.
in two of the growth solutions, consistent with $\varepsilon^{15}$N values around -1.5 ‰ and $\varepsilon^{15}$N/$\varepsilon^{18}$O of around 0.5 – 0.6 might have resulted from isotopic fractionation during uptake into the plants, or they might indicate microbial reduction of NO$_3^-$ in solution. If bacterial NO$_3^-$ reduction occurred, it does not appear to have been accompanied by substantial ClO$_4^-$ reduction based on ClO$_4^-$ isotopic compositions.

Although there was a substantial mass of ClO$_4^-$ (27-42%) not accounted for in experiment 1, the lack of change in solution ClO$_4^-$ stable isotopic composition compared to source material indicates ClO$_4^-$ was not a major sink for ClO$_4^-$ in growth solutions. If fractionation of ClO$_4^-$ occurred during uptake, then the magnitude was too small to detect in these experiments and unlikely to be a significant factor for the variation in ClO$_4^-$ stable isotopic composition observed in natural systems. However, it is possible that hydroponic studies may not fully reflect uptake processes in normal soil plant systems.

Plant-mediated transformation of ClO$_4^-$ could also cause mass-dependent fractionation of ClO$_4^-$.

In contrast to ClO$_4^-$, $\delta^{15}$N of NO$_3^-$ in plant tissue was fractionated substantially (~10-20‰), consistent with reported fractionation effects of NO$_3^-$ assimilation in marine culture studies (~15-30‰) (Needoba et al., 2004; Granger et al., 2010; Ledgard et al., 2005; Schmidt and Medina, 1991). The $\varepsilon^{15}$N/$\varepsilon^{18}$O ratios of 1.04 – 1.07 observed in our study support previous experimental studies indicating that isotopic fractionation by assimilatory nitrate reductase has an intrinsic $\varepsilon^{15}$N/$\varepsilon^{18}$O ratio of approximately 1, regardless of the magnitudes of $\varepsilon^{15}$N and $\varepsilon^{18}$O values expressed (Karsh et al., 2012, 2014; Granger et al., 2004).

We also demonstrate that field-grown plants exposed to environmentally relevant ClO$_4^-$ concentrations do not appear to affect foliar ClO$_4^-$ isotopic composition. ClO$_4^-$ extracted from snap beans grown in Raleigh varied somewhat isotopic composition between 2009 and 2010 based presumably on the source of irrigation water, but the stable isotope data clearly showed a
significant component of Chilean-type ClO₄⁻, which was also the predominant source in local groundwater based on isotopic analysis. Commercial spinach had an isotopic composition similar to that of indigenous natural ClO₄⁻ from the SHP of West Texas and New Mexico as well as the U.S Great Lakes (Sections 2.2 & 2.3; Jackson et al., 2010; Böhlke et al., 2005; Hatzinger et al., 2015; Sturchio et al., 2006; Sturchio et al., 2014; Poghosyan et al., 2014). This result could indicate the spinach was exposed to natural ClO₄⁻ with a similar isotopic composition in soil or irrigation water in one or both of the potential source areas of the spinach (Arizona and southwestern California). The lower concentrations of ClO₄⁻ in spinach, compared to those in the hydroponic bean plants, are consistent with exposure to low level background ClO₄⁻ commonly found in groundwater (MRGB: 0.12 to 0.12 to 1.8µg/L; SHP: ~0.1 to 200 µg/L) or soil (SHP: 3.3µg/kg) (Jackson et al., 2010). Because the spinach ClO₄⁻ time of exposure likely was similar to time of plant exposure in the hydroponic studies, it might be concluded that time-related isotope effects were minimal and the spinach ClO₄⁻ isotopic composition represents the source ClO₄⁻ isotopic composition. However, it is also possible that ClO₄⁻ in spinach tissue was altered isotopically (e.g. reduction or oxygen exchange). If so, while the O isotopic composition of spinach ClO₄⁻ might be relatively independent of the source values, the Cl isotopic composition of spinach ClO₄⁻ might still reflect the source value, which would be consistent with an indigenous ClO₄⁻ source and not electrochemical or Atacama ClO₄⁻. Although only one composite sample of spinach was extracted, these results combined with those of the field and hydroponic studies suggest that it should be possible to evaluate the source of ClO₄⁻ in commercial produce and in other plant-based food products. This finding is important because most of the exposure to ClO₄⁻ in the U.S. population and likely the populations of many other countries is through ingestion of produce (Jackson et al., 2005, El Aribi et al., 2006, Sanchez et al., 2005a,b). Thus, isotopic ratio measurements of ClO₄⁻ in plant tissue may provide a direct method to distinguish the dominant sources of ClO₄⁻ in the U.S. and other food chains worldwide.

It is possible that hydroponic studies may not fully reflect uptake processes in normal soil-plant systems and that snap beans may not represent other plant types. However, our results indicate ClO₄⁻ uptake and accumulation can occur without significant net loss or isotope effects and that plant NO₃⁻ reduction systems efficiently exclude ClO₄⁻, in contrast to some other NO₃⁻ reducing systems, such as bacteria (Hatzinger et al., 2009; Nerenberg et al., 2008; Maixner et al., 2008; Chaudhuri et al., 2002). These results suggest that isotopic characteristics of ClO₄⁻ in soils and groundwater may not be affected by plant uptake and release. Therefore, our results do not support the hypothesis that plant uptake is a major cause of variation in ClO₄⁻ stable isotopic composition observed in natural systems. Given the relatively large contribution of U.S. human exposure through food, further studies may be warranted to determine sources of ClO₄⁻ in the food chain, as well as to determine if other types of plants in other settings can alter the isotopic composition of ClO₄⁻.
2.9 Publications

Some of the data and text presented herein was previously published in the following journal articles and dissertations. Sections from these articles are reprinted with permission as required.


3.0 CONCLUSIONS and IMPLICATIONS FOR FUTURE RESEARCH

The results from a range of different laboratory studies evaluating ClO$_4^-$ formation mechanisms confirm that there are multiple potential pathways of ClO$_4^-$ generation from both UV-photolysis and O$_3$-mediated oxidation of Cl$^-$ and other ClO$_x$ precursors. Laboratory studies were successful in producing Cl and O isotopic variations in ClO$_4^-$ that incorporate much of the reported stable isotope variation in natural ClO$_4^-$. Only the characteristically low $\delta^{37}$Cl values of Atacama ClO$_4^-$ (Chile) were not reproduced and these remain enigmatic. Data indicate that final ClO$_4^-$ isotopic composition is dependent on the precursor species oxidized. The reaction rates and intermediate species proposed to be involved in ClO$_4^-$ formation require further study and additional experiments are required to resolve the reason for the low $\delta^{37}$Cl values of Atacama ClO$_4^-$, but significant progress was made in constraining pathways of ClO$_4^-$ production in nature by application of stable isotope analysis.

Worldwide soil and groundwater sampling data indicate that ClO$_4^-$ is globally distributed in soil and groundwater in arid and semi-arid regions on Earth at concentrations ranging from $10^{-1}$ to $10^6$ µg/kg. Generally, the ClO$_4^-$ concentration in these regions increases with aridity index, but this also depends on the duration of arid conditions. In many arid and semi-arid areas, NO$_3^-$ and ClO$_4^-$ co-occur at consistent ratios (NO$_3^-$/ClO$_4^-$) that vary between $\sim10^4$ and $\sim10^5$. This is not the case for Cl$^-$/ClO$_4^-$ ratios, which vary widely among locations. The NO$_3^-$/ClO$_4^-$ ratios are largely preserved in hyper-arid areas that support little or no biological activity (e.g. plants or bacteria), but can be altered in areas with more active biological processes. In general, the co-occurrence of ClO$_4^-$ and NO$_3^-$ in arid and semi-arid locations, and associated variations in the isotopic composition of the NO$_3^-$, are consistent with a conceptual model of atmospheric origin, global co-deposition, and variable alteration of the NO$_3^-$ pool by biogenic addition, assimilation, and/or recycling on the surface. The Atacama Desert appears to be unique compared to other arid and semi-arid locations. There, exceptional enrichment in ClO$_4^-$ compared to Cl$^-$ or NO$_3^-$, accompanied by unique ClO$_4^-$ isotopic characteristics, may reflect an unusually efficient, but yet unknown, in situ production mechanism, regionally elevated atmospheric ClO$_4^-$ production rates, or higher ClO$_4^-$ production rates in pre-Pleistocene times. Further laboratory and field research is required to better understand and identify the conditions and/or processes that have led to the unique ClO$_4^-$ isotopic characteristics and high relative ClO$_4^-$ concentrations in the Atacama.

Stable isotope analysis of Cl and O and radioactive isotope analysis of $^{36}$Cl in natural ClO$_4^-$ confirmed and extended initial data suggesting that indigenous ClO$_4^-$ sources in the southwestern U.S. show some isotopic variation by location and environment but remain isotopically distinct from synthetic and Atacama ClO$_4^-$ when all relevant isotope ratios are considered. Perchlorate concentration and isotope analysis was conducted in all five of the North American Great Lakes. The data showed average ClO$_4^-$ concentrations ranging from 0.05 to 0.13 µg/L (varying by lake) with concentrations being nearly constant with depth. Interestingly, the overall ranges of stable isotopic compositions of Great Lakes ClO$_4^-$ resemble those of indigenous natural ClO$_4^-$ measured
in groundwaters of the western USA indicating a predominantly natural atmospheric source of ClO$_4^-$ in all of the lakes.

ClO$_4^-$ and ClO$_3^-$ concentration profiles with depth were also collected from ice-covered lakes in the McMurdo Dry Valleys MDV of Antarctica. Sample quantities, however, were insufficient for stable isotope analysis. These lakes provide an excellent case study for ClO$_3^-$ and ClO$_4^-$ biotransformation in pristine extreme environments. Given their low concentrations, high solubility, and lack of any in situ generation mechanisms, ClO$_3^-$ and ClO$_4^-$ may offer a sensitive means to study ongoing biological activity in the lakes, and the addition of ClO$_4^-$ stable isotope evaluation could provide further clues as to the geochemical history of the lake water. Finally, ClO$_3^-$ and ClO$_4^-$ biogeochemistry in Antarctic ice-covered lakes may represent an excellent analog for similar processes in ice-covered lakes on Mars in the past or even in more recent times, especially given the discovery of relatively large amounts of ClO$_4^-$ in the Martian soil. Further research with Antarctic ClO$_4^-$ samples, including isotopic analysis is recommended.

Several potential biological mechanisms of ClO$_4^-$ generation were evaluated to determine if any could be a secondary source of this anion in the environment and to help explain the isotopic characteristics and variation in some natural ClO$_4^-$ samples. Bacterial production of ClO$_4^-$ was assessed using (1) various nitrifying cultures and enrichments that oxidize NH$_4$ to NO$_3^-$; (2) natural haloperoxidase enzymes that are known to oxidize Cl$^-$ to hypochlorous acid (HClO) and potentially to ClO$_4^-$ (possibly via additional photochemical or biological reactions); and (3) organisms capable of oxidizing sulfite or phosphite. A variety of experiments were conducted with Cl$^-$ or ClO$_3^-$ precursors in the presence of different enzymes, organisms, and conditions as summarized above. While some ClO$_4^-$ generation was initially indicated via haloperoxidase enzymes in the presence of UV light, this result was not consistent and is unlikely to account for significant ClO$_4^-$ production. The other organisms and processes evaluated did not result in ClO$_4^-$ formation.

A variety of plant species were also evaluated for their potential to accumulate and even generate ClO$_4^-$ via O$_3$-mediated processes. A broad range of crop species was observed to accumulate ClO$_4^-$ from growth medium, and these species differed widely in their bioconcentration of the anion. 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, there was no evidence that exposure of leaves to ambient O$_3$ or at significantly elevated O$_3$ induced any increase in tissue contents of ClO$_4^-$.

The results indicate that O$_3$ does not lead to increased phyto-accumulation nor plant biosynthesis of ClO$_4^-$.

The impact of plant accumulation of ClO$_4^-$ on Cl and O stable isotope values was also evaluated in both hydroponic laboratory studies and field crops grown in different parts of the U.S. In hydroponic studies with snap beans, no substantial differences were observed in the $\delta^{37}$Cl, $\delta^{18}$O, of $\Delta^{17}$O values of ClO$_4^-$ between the growth solutions and leaf extracts. In contrast to ClO$_4^-$, $\delta^{15}$N
of NO$_3^-$ in plant tissue was fractionated substantially (~10-20%). The $\varepsilon^{15}$N/$\varepsilon^{18}$O ratios of 1.04 – 1.07 support previous experimental studies showing similar ratios via assimilatory nitrate reductase. The data indicate that plants do not metabolize and assimilate ClO$_4^-$ similarly to NO$_3^-$. 

Similar to hydroponically grown plants, field grown plants exposed to environmentally relevant ClO$_4^-$ concentrations also did not appear to affect foliar ClO$_4^-$ isotopic composition. ClO$_4^-$ extracted from snap beans grown in Raleigh, NC varied somewhat in isotopic composition between two growing seasons based presumably on the source of irrigation water, but the stable isotope data clearly showed a significant component of Atacama-type ClO$_4^-$ from past fertilizer use which was also the predominant source in local groundwater based on isotopic analysis. Commercial spinach was also extracted and analyzed for ClO$_4^-$ stable isotopes. The spinach had an isotopic composition similar to that of indigenous natural ClO$_4^-$ from the SHP as well as the Great Lakes. This result could indicate the spinach was exposed to natural ClO$_4^-$ with a similar isotopic composition in soil or irrigation water in one or both of the potential source areas of the spinach (Arizona and southwestern California). Although this spinach that was extracted represents only one composite sample, these results combined with those of the field bean and hydroponic studies suggest that it should be possible to evaluate the dominant source of ClO$_4^-$ (i.e., synthetic, Atacama, indigenous) in commercial produce and in other plant-based food products through stable isotope analysis of plant accumulated ClO$_4^-$. This finding is important because most of the exposure to ClO$_4^-$ in the U.S. population and likely the populations of many other countries is through ingestion of produce. Additional research is warranted with a variety of food crops and products to confirm that ClO$_4^-$ isotope signatures in vegetation reflect those in irrigation water and/or local soils and groundwater, thus allowing forensic determination of ClO$_4^-$ sources in food crops.

Overall, the results of this project have provided important new information on natural ClO$_4^-$ in the environment. Significant progress was made concerning potential mechanisms of its formation, isotopic characterization of natural ClO$_4^-$ sources in groundwater, lakes, soils and plants, and its worldwide occurrence and accumulation in arid and semi-arid environments compared with that of NO$_3^-$, Cl$^-$, and other anions. The data support previous studies showing that natural and synthetic ClO$_4^-$ can be differentiated by stable isotope methods, and suggest for the first time that the source(s) of ClO$_4^-$ in food crops may be determined by isotopic analysis of ClO$_4^-$ in plant tissue. While this project has provided a much more comprehensive understanding of natural ClO$_4^-$, significant research questions still remain, including whether natural ClO$_4^-$ production mechanisms exist other than the UV and O$_3$ mediated processes identified in this and other studies, and whether isotopic exchange processes exist that alter the stable isotope signatures of produced ClO$_4^-$. A more thorough understanding of these factors could resolve remaining uncertainties concerning the observed range of stable isotope signatures of natural ClO$_4^-$, including the seemingly unique isotopic characteristics and relative concentrations of ClO$_4^-$ in the Atacama Desert.
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Appendix A: Proposed Mechanisms of Perchlorate Formation
A.1 Proposed Pathways for O₃ Oxidation of Aqueous Chloride

A.1.1 ClO₄⁻ formed from ClO₃ oxidation by OH radical (Final Proposed Pathways)

Figure A.1 Mechanism 1: O₃ oxidation of Cl⁻ (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing all four O’s from O₃.
Figure A.2 Mechanism 2: O$_3$ oxidation of Cl$^-$ (aq). Note the thick pink lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule one containing four O’s from O$_3$. 

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Figure A.3 Mechanism 3. O₃ oxidation of Cl⁻ (aq). Note the thick aqua blue lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing all four O’s from O₃.
Figure A.4  Mechanism 4. O₃ oxidation of Cl⁻ (aq). Note the thick orange lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing all four O’s from O₃.
A.1.2 ClO₄⁻ Formed from Hydrolysis of the Cl₂O₇ Species

Figure A.5 Mechanism 5: O₃ oxidation of Cl⁻ (aq). Note the thick red lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two ClO₄⁻ molecules, one with three O from O₃ and the other with four O from O₃.
Figure A.6 Mechanism 6: O₃ oxidation of Cl⁻ (aq). Note the thick blue lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two ClO₄⁻ molecules one containing three O’s from O₃ and the other four O’s from O₃.
Figure A.7 Mechanism 7. O₃ oxidation of Cl⁻ (aq). Note the thick apple green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two ClO₄⁻ molecules, one with three O from O₃ and the other with four O from O₃.
Figure A.8 Mechanism 8. O₃ oxidation of Cl⁻ (aq). Note the thick light blue lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two ClO₄⁻ molecules, one with three O from O₃ and the other with four O from O₃.
A.1.3 Pathways that Produce ClO$_4^-$ with Only 3 O atoms from O$_3$

Figure A.9 Mechanism 9: O$_3$ oxidation of Cl$^-$ (aq). Note the thick blue lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule containing three O’s from O$_3$ and a ClO$_3^-$ molecule containing all three O’s from O$_3$. 
Figure A.10  Mechanism 10: O₃ oxidation of Cl⁻ (aq). Note the thick green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.11 Mechanism 11: O₃ oxidation of Cl⁻ (aq). Note the thick orange lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.12 Mechanism 12: O₃ oxidation of Cl⁻ (aq). Note the thick aquamarine lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.13 Mechanism 13: O₃ oxidation of Cl⁻ (aq). Note the thick maroon lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃ and a ClO₃⁻ molecule containing all three O’s from O₃.
Figure A.14 Mechanism 14: O₃ oxidation of Cl⁻ (aq). Note the thick magenta lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.15 Mechanism 15: O₃ oxidation of Cl⁻ (aq). Note the thick olive green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.16  Mechanism 16.  O₃ oxidation of Cl⁻ (aq).  Note the thick dark cyan lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4.  This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.17 Mechanism 17. O₃ oxidation of Cl⁻ (aq). Note the thick dark blue lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃ and a ClO₃⁻ molecule containing all three O’s from O₃.
Figure A.18 Mechanism 18. O₃ oxidation of Cl⁻ (aq). Note the thick apricot lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.19 Mechanism 19. O₃ oxidation of Cl⁻ (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.20  Mechanism 20. O₃ oxidation of Cl⁻ (aq). Note the thick lime green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.21  Mechanism 21. O₃ oxidation of Cl⁻ (aq). Note the blue lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃ and a ClO₃⁻ molecule containing all three O’s from O₃.
Figure A.22  Mechanism 22. O₃ oxidation of Cl⁻ (aq). Note the pink lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.23  Mechanism 23. O$_3$ oxidation of Cl$^-$ (aq). Note the grayish purple lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule containing three O’s from O$_3$. 
Figure A.24  Mechanism 24. O₃ oxidation of Cl⁻ (aq). Note the thick red lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.25 Mechanism 25. O₃ oxidation of Cl⁻ (aq). Note the thick red lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.26  Mechanism 26. $O_3$ oxidation of $Cl^-$ (aq). Note the thick forest green lines indicate the pathway leading to the formation of the $ClO_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one $ClO_4^-$ molecule containing three O’s from $O_3$. 
A.2 Proposed Pathways for O₃ Oxidation of Hypochlorite (aq)

A.2.1 Pathways that Produce ClO₄⁻ with only three O’s from O₃

Figure A.27  Mechanism 1:  O₃ oxidation of OCl⁻ (aq).  Note the thick red lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4.  This pathway leads to the formation of one ClO₄⁻ molecule with three O’s from O₃.
Figure A.28  Mechanism 2: O₃ oxidation of OCl⁻ (aq). Note the thick red lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.29 Mechanism 3: O₃ oxidation of OCl⁻ (aq). Note the thick green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing three O’s from O₃.
Figure A.30 Mechanism 4: O$_3$ oxidation of OCl$^-$ (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule containing three O’s from O$_3$. 
A.2.1 Pathways that Produce ClO$_4^-$ with two and three O’s from O$_3$

Figure A.31  Mechanism 5:  O$_3$ oxidation of OCl$^-$ (aq).  Note the thick dark aqua lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4.  This pathway leads to the formation of two ClO$_4^-$ molecules, one with three O from O$_3$ and the other with two O from O$_3$. 
Figure A.32 Mechanism 6: \( \text{O}_3 \) oxidation of \( \text{OCl}^- \) (aq). Note the thick dark pink lines indicate the pathway leading to the formation of the \( \text{ClO}_4^- \) and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two \( \text{ClO}_4^- \) molecules, one with three \( \text{O} \) from \( \text{O}_3 \) and the other with two \( \text{O} \) from \( \text{O}_3 \).
Figure A.33 Mechanism 7: O₃ oxidation of OCl⁻ (aq). Note the thick dark purple lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two ClO₄⁻ molecules, one with three O from O₃ and the other with two O from O₃.
Figure A.34 Mechanism 8: O₃ oxidation of OCl⁻ (aq). Note the thick blue lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two ClO₄⁻ molecules, one with three O from O₃ and the other with two O from O₃.
A.2.2 Pathways that Produce ClO$_4^-$ with only two O’s from O$_3$

Figure A.35 Mechanism 9: O$_3$ oxidation of OC(I) (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule containing two O’s from O$_3$. 
Figure A.36 Mechanism 10: O$_3$ oxidation of OCl$^-$ (aq). Note the thick blue lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule containing two O’s from O$_3$. 
Figure A.37 Mechanism 11: \( \text{O}_3 \) oxidation of \( \text{OCl}^- \) (aq). Note the thick yellow lines indicate the pathway leading to the formation of the \( \text{ClO}_4^- \) and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one \( \text{ClO}_4^- \) molecule containing two O’s from \( \text{O}_3 \).
Figure A.38 Mechanism 12: O₃ oxidation of OCl⁻ (aq). Note the thick dark green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing two O’s from O₃.
Figure A.39 Mechanism 13: O₃ oxidation of OCl⁻ (aq). Note the thick aqua green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing two O’s from O₃.
A.2.3. Pathways involving the Cl₂O₅ Species

Figure A.40  Mechanism 14: O₃ oxidation of OCl⁻ (aq). Note the thick neon green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing two O’s from O₃.
Figure A.41  Mechanism 15: O₃ oxidation of OCl⁻ (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing two O’s from O₃.
Figure A.42 Mechanism 16: O$_3$ oxidation of OC$^-$ (aq). Note the thick red lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule containing two O’s from O$_3$. 
Figure A.43 Mechanism 17: $O_3$ oxidation of $OCl^-$ (aq). Note the thick purple lines indicate the pathway leading to the formation of the $ClO_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one $ClO_4^-$ molecule containing two O’s from $O_3$. 
A.2.4 Pathways Involving Speculated Reactions 16 and 19

Figure A.44  Mechanism 18: O$_3$ oxidation of OCl$^-$ (aq). Note the thick blue green lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule containing two O’s from O$_3$. 
Figure A.45  Mechanism 19: $\text{O}_3$ oxidation of $\text{OCl}^-$ (aq). Note the thick gray lines indicate the pathway leading to the formation of the $\text{ClO}_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one $\text{ClO}_4^-$ molecule containing two O’s from $\text{O}_3$. 
Figure A.46 Mechanism 20: $O_3$ oxidation of $OCl^-$ (aq). Note the thick red lines indicate the pathway leading to the formation of the $ClO_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one $ClO_4^-$ molecule containing two O’s from $O_3$. 
Figure A.47 Mechanism 21: O₃ oxidation of OCl⁻ (aq). Note the thick orange lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO₄⁻ molecule containing two O’s from O₃.
Figure A.48 Mechanism 22: $O_3$ oxidation of $OCl^-$ (aq). Note the thick blue lines indicate the pathway leading to the formation of the $ClO_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one $ClO_4^-$ molecule containing two O’s from $O_3$. 
Figure A.49  Mechanism 23: O$_3$ oxidation of OCl$^-$ (aq). Note the thick aqua lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO$_4^-$ molecule containing two O’s from O$_3$. 
A.3 Proposed Pathways for O₃ Oxidation of Aqueous ClO₂⁻

A.3.1 Pathways Producing a ClO₄⁻ with only two O from O₃

Figure A.50  Mechanism 1: O₃ oxidation of ClO₂⁻ (aq). Note the thick orange lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO₄⁻ molecule containing two O from O₃.
A.3.2 Pathways Producing two ClO$_4^-$ molecules, one with only one O from O$_3$ and the other with two

Figure A.51  Mechanism 2: O$_3$ oxidation of ClO$_2^-$ (aq). Note the thick maroon lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two ClO$_4^-$ molecules one containing one O from O$_3$ and the other two O’s from O$_3$. 
A.3.3 Pathways Producing ClO$_4^-$ with only one O from O$_3$.

Figure A.52  Mechanism 3: O$_3$ oxidation of ClO$_2^-$ (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO$_4^-$ molecule containing one O from O$_3$. 
Figure A.53 Mechanism 4: O₃ oxidation of ClO₂⁻ (aq). Note the thick light blue lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO₄⁻ molecule containing one O from O₃ and one ClO₃⁻ molecule containing one O from O₃.
A.3.4 Pathways Involving the Cl\textsubscript{2}O\textsubscript{5} species

Figure A.54  Mechanism 5: O\textsubscript{3} oxidation of ClO\textsuperscript{2-} (aq). Note the thick green lines indicate the pathway leading to the formation of the ClO\textsubscript{4}\textsuperscript{-} and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO\textsubscript{4}\textsuperscript{-} molecule containing one O from O\textsubscript{3}.
A.3.5 Pathways Involving Reactions 16 and 19

Figure A.55  Mechanism 6: O₃ oxidation of ClO₂⁻ (aq). Note the thick orange lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO₄⁻ molecule containing one O from O₃.
A.4 Proposed Pathways for O$_3$ Oxidation of Aqueous ClO$_2$

A.4.1 Pathways Producing a ClO$_4^-$ with only two O from O$_3$

Figure A.56  Mechanism 1: O$_3$ oxidation of ClO$_2$ (aq). Note the thick maroon lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO$_4^-$ molecule containing two O from O$_3$. 

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A.4.2 Pathways Producing a ClO$_4^-$ with one or two O from O$_3$

Figure A.57 Mechanism 2: O$_3$ oxidation of ClO$_2$ (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two ClO$_4^-$ molecules one containing one O from O$_3$ and the other two O’s from O$_3$. 
A.4.3 Pathways Producing a ClO$_4^-$ with only one O from O$_3$.

Figure A.58 Mechanism 3: O$_3$ oxidation of ClO$_2$ (aq). Note the thick pink lines indicate the pathway leading to the formation of the ClO$_4^-$ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO$_4^-$ molecule containing one O from O$_3$ and one ClO$_3^-$ molecule containing one O from O$_3$. 
Figure A.59 Mechanism 4: O₃ oxidation of ClO₂ (aq). Note the thick green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO₄⁻ molecule containing one O from O₃.
Figure A.60 Mechanism 5: O₃ oxidation of ClO₂ (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO₄⁻ molecule containing one O from O₃.
Figure A.61  Mechanism 6: O₃ oxidation of ClO₂ (aq). Note the thick green lines indicate the pathway leading to the formation of the ClO₄⁻ and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO₄⁻ molecule containing one O from O₃.