

# FINAL REPORT

## Phase II: Identification and Characterization of Natural Sources of Perchlorate

SERDP Project ER-1435

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| 14. ABSTRACT<br>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. |                  |                         |                                         |                                                                |                                                           |
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**Figure 2.5.19.** Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq).

**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).

**Figure 2.5.21.** Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  (aq).

**Figure 2.5.22.** Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  including possible pathways for  $\text{ClO}_3^-$  formation (aq).

**Figure 2.5.23.**  $\text{O}_3$  oxidation of  $\text{ClO}_2$  (aq). Note the thick maroon 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 one  $\text{ClO}_4^-$  molecule containing two O from  $\text{O}_3$ .

**Figure 2.5.24.** UV oxidation of  $\text{OCl}^-$  (aq).

**Figure 2.5.25.** UV oxidation of  $\text{ClO}_2^-$  (aq).

**Figure 2.5.26.** UV oxidation of  $\text{ClO}_2$  (aq).

**Figure 2.5.27.** Stable O isotopic compositions ( $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) of reactant species and final  $\text{ClO}_4^-$  for  $\text{O}_3$  oxidized  $\text{ClO}_x$  solutions including expected  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  of  $\text{ClO}_4^-$  formed from the mixture of  $\text{H}_2\text{O}$  and  $\text{O}_3$  (with complete site preference of the heavy O isotope in the terminal O atoms of  $\text{O}_3$ ) (black circles) and expected  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  of  $\text{ClO}_4^-$  formed from the mixture of  $\text{H}_2\text{O}$  and  $\text{O}_3$  (with no site preference of the heavy O isotope in the terminal O atoms of  $\text{O}_3$ ) (green circles).

**Figure 2.5.28.** Summary of isotope data for  $\text{ClO}_4^-$  produced in the  $\text{O}_3$  (square symbols) and UV (circle symbols) generation experiments.

**Figure 2.6.1.** Results of MCD assays.

**Figure 2.7.1.** Inter-specific differences in perchlorate content ( $\mu\text{g (kg dry wt)}^{-1}$ ) of youngest fully expanded leaves of a range of crop species (mean over all  $\text{O}_3$  exposures  $\pm$  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  $\text{O}_3$ ) and the change in  $\text{ClO}_4^-$  content (unitless) between 4 and 59 ppb  $\text{O}_3$  (circles) and between 4 and 114 ppb (squares) of youngest fully expanded leaves of a range of crop species.

**Figure 2.7.3.** There was no significant relationship between  $\text{O}_3$  exposure (A;  $r^2 = 0.0034$ ) or  $\text{O}_3$  dose (cumulative flux; B;  $r^2=0.0018$ ) and  $\text{ClO}_4^-$  content of young leaves normalized by the median  $\text{ClO}_4^-$  content of each species shown in Figure 2.7.1 for which  $\text{O}_3$  flux data were available.

**Figure 2.7.4.** Significant relationship between perchlorate concentration ( $\mu\text{g (kg dry wt)}^{-1}$ ) of potting medium exposed in the CSTRs and  $\text{O}_3$  exposure ( $\text{nL L}^{-1}$ ; 12 hr mean;  $r^2 = 0.99$ ;  $P = 0.0714$ ).

**Figure 2.7.5.** Relationship between perchlorate concentration ( $\mu\text{g (kg dry wt)}^{-1}$ ) of young leaves and  $\text{O}_3$  exposure.

**Figure 2.7.6.** Effect of  $\text{O}_3$  exposure on stomatal conductance ( $g_s$ ).

**Figure 2.7.7.** Relationship between (A) perchlorate concentration of young leaves ( $\mu\text{g (kg dry wt)}^{-1}$ ) and  $\text{O}_3$  dose; or (B) perchlorate concentration (unitless) normalized by the median concentration over all  $\text{O}_3$  within each species, and  $\text{O}_3$  dose.

**Figure 2.8.1.** Cumulative volume of growth solution uptaken by pooled replicate treatments in both experiments.

**Figure 2.8.2.** Fraction of  $\text{ClO}_4^-$ ,  $\text{Cl}^-$ , and  $\text{NO}_3^-$  mass remaining in nutrient solutions at the time of harvest.

**Figure 2.8.3.** Relationship between fraction of total (a)  $\text{ClO}_4^-$ , (b)  $\text{Cl}^-$ , and (c)  $\text{NO}_3^-$  mass lost from solution and cumulative volume of solution transpired over the course of experiment 2.

**Figure 2.8.4.** Stable  $\text{ClO}_4^-$  isotopic composition of nutrient solutions and leaf extracts of replicate treatments in experiment 1 and 2.

**Figure 2.8.5.** Stable isotopic composition of  $\text{NO}_3^-$  in nutrient solutions as a function of time for experiment 1 (a, b) and experiment 2 (d, e).

**Figure 2.8.6.** Rayleigh plots of the change in the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  in nutrient solutions of experiment 1(a, b, c) and experiment 2 (d, e, f). =F

**Figure 2.8.7.** Stable isotopic composition of  $\text{NO}_3^-$  in final growth solutions (solid symbols) and leaf extracts (open symbols) of pooled replicate treatments in experiments 1 and 2.

**Figure 2.8.8.** Summary of isotope data for  $\text{ClO}_4^-$  from Raleigh, NC and Long Island, NY field studies and commercial spinach displayed with previously published  $\text{ClO}_4^-$  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).



## ACRONYM LIST

### Acronyms and Abbreviations

|                                |                                                        |
|--------------------------------|--------------------------------------------------------|
| ‰                              | per mil                                                |
| $\delta$ , $\Delta$            | delta, relative difference of isotope ratios           |
| ACS                            | American Chemical Society                              |
| ANOVA                          | Analysis of Variance                                   |
| Ag                             | silver                                                 |
| AgCl                           | silver chloride                                        |
| AgNO <sub>3</sub>              | silver nitrate                                         |
| Al                             | aluminum                                               |
| Al <sub>2</sub> O <sub>3</sub> | activated alumina                                      |
| AMS                            | accelerator mass spectrometry                          |
| Aq                             | aqueous                                                |
| Ar                             | argon                                                  |
| Br <sup>-</sup>                | 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        |
| CH <sub>4</sub>                | methane                                                |
| CH <sub>3</sub> Cl             | methyl chloride                                        |
| CH <sub>3</sub> I              | methyl iodide                                          |
| Cl <sup>-</sup>                | chloride                                               |
| Cl                             | chlorine                                               |
| <sup>35</sup> Cl               | chlorine-35                                            |
| <sup>36</sup> Cl               | chlorine-36                                            |
| <sup>37</sup> Cl               | chlorine-37                                            |
| ClO <sub>2</sub>               | chlorine dioxide                                       |
| ClO <sub>2</sub> <sup>-</sup>  | chlorite                                               |
| ClO <sub>3</sub> <sup>-</sup>  | chlorate                                               |
| ClO <sub>4</sub> <sup>-</sup>  | perchlorate                                            |
| ClO <sub>x</sub>               | oxychlorine species                                    |
| cm                             | centimeters                                            |
| CO                             | carbon monoxide                                        |
| CO <sub>2</sub>                | carbon dioxide                                         |
| COC                            | chain of custody                                       |
| CsCl                           | cesium chloride                                        |
| CsClO <sub>4</sub>             | cesium perchlorate                                     |
| CsOH                           | cesium hydroxide                                       |

|                                     |                                                                          |
|-------------------------------------|--------------------------------------------------------------------------|
| <b>CSTR</b>                         | <b>continuously stirred tank reactor</b>                                 |
| <b>DDI</b>                          | <b>distilled deionized water</b>                                         |
| <b>DI</b>                           | <b>deionized water</b>                                                   |
| <b>DI-IRMS</b>                      | <b>dual-inlet isotope ratio mass spectrometry</b>                        |
| <b>DO</b>                           | <b>dissolved oxygen</b>                                                  |
| <b>DOC</b>                          | <b>dissolved organic carbon</b>                                          |
| <b>DoD</b>                          | <b>Department of Defense</b>                                             |
| <b>EL</b>                           | <b>East Lobe Lake Bonney, Antarctica</b>                                 |
| <b>EPA</b>                          | <b>Environmental Protection Agency</b>                                   |
| <b>ESTCP</b>                        | <b>Environmental Security Technology Certification Program</b>           |
| <b>Fe<sup>3+</sup></b>              | <b>Iron (III)</b>                                                        |
| <b>FeCl<sub>3</sub></b>             | <b>iron (III) chloride</b>                                               |
| <b>FeCl<sub>4</sub><sup>-</sup></b> | <b>tetrachloroferrate</b>                                                |
| <b>Fe(OH)<sub>3</sub></b>           | <b>iron (III) hydroxide</b>                                              |
| <b>ft</b>                           | <b>feet</b>                                                              |
| <b>FW</b>                           | <b>fresh weight</b>                                                      |
| <b>g</b>                            | <b>gram</b>                                                              |
| <b>gal</b>                          | <b>gallon</b>                                                            |
| <b>GC</b>                           | <b>gas chromatograph</b>                                                 |
| <b>g<sub>s</sub></b>                | <b>stomatal conductance</b>                                              |
| <b><sup>3</sup>H</b>                | <b>tritium</b>                                                           |
| <b>ha</b>                           | <b>hectare</b>                                                           |
| <b>HCl</b>                          | <b>hydrochloric acid</b>                                                 |
| <b>HClO<sub>4</sub></b>             | <b>perchloric acid</b>                                                   |
| <b>HCO<sub>3</sub></b>              | <b>bicarbonate</b>                                                       |
| <b>He</b>                           | <b>helium</b>                                                            |
| <b>HNO<sub>3</sub></b>              | <b>nitric acid</b>                                                       |
| <b>H<sub>2</sub>O</b>               | <b>water</b>                                                             |
| <b>H<sub>2</sub>O<sub>2</sub></b>   | <b>hydrogen peroxide</b>                                                 |
| <b>HPLC-UV</b>                      | <b>high performance liquid chromatography with ultraviolet detection</b> |
| <b>hr</b>                           | <b>hours</b>                                                             |
| <b>H<sub>2</sub>SO<sub>4</sub></b>  | <b>sulfuric acid</b>                                                     |
| <b>IAEA</b>                         | <b>International Atomic Energy Agency</b>                                |
| <b>IC</b>                           | <b>ion chromatography</b>                                                |
| <b>IO<sub>3</sub><sup>-</sup></b>   | <b>iodate</b>                                                            |
| <b>IRMS</b>                         | <b>isotope-ratio mass-spectrometry</b>                                   |
| <b>ITRC</b>                         | <b>Interstate Technology &amp; Regulatory Council</b>                    |
| <b>IX</b>                           | <b>ion exchange</b>                                                      |
| <b>k</b>                            | <b>one thousand</b>                                                      |
| <b>K</b>                            | <b>potassium</b>                                                         |
| <b>KCl</b>                          | <b>potassium chloride</b>                                                |
| <b>KClO<sub>4</sub></b>             | <b>potassium perchlorate</b>                                             |
| <b>kg</b>                           | <b>kilogram</b>                                                          |
| <b>km<sup>2</sup></b>               | <b>square kilometer</b>                                                  |

|                                                 |                                                                       |
|-------------------------------------------------|-----------------------------------------------------------------------|
| <b>KNO<sub>3</sub></b>                          | <b>potassium nitrate</b>                                              |
| <b>KOH</b>                                      | <b>potassium hydroxide</b>                                            |
| <b>L</b>                                        | <b>liter</b>                                                          |
| <b>LUB</b>                                      | <b>Lower Umatilla Basin</b>                                           |
| <b>LC-MS/MS</b>                                 | <b>liquid chromatography-tandem mass spectrometry</b>                 |
| <b>µg</b>                                       | <b>microgram</b>                                                      |
| <b>µg/L</b>                                     | <b>microgram per liter</b>                                            |
| <b>µm</b>                                       | <b>micron</b>                                                         |
| <b>µmol</b>                                     | <b>micromole</b>                                                      |
| <b>M</b>                                        | <b>molar</b>                                                          |
| <b>m</b>                                        | <b>meter</b>                                                          |
| <b>MDL</b>                                      | <b>method detection limit</b>                                         |
| <b>MDV</b>                                      | <b>McMurdo Dry Valleys, Antarctica</b>                                |
| <b>Mg</b>                                       | <b>magnesium</b>                                                      |
| <b>mg</b>                                       | <b>milligram</b>                                                      |
| <b>MRGB</b>                                     | <b>Middle Rio Grande Basin</b>                                        |
| <b>min</b>                                      | <b>minute</b>                                                         |
| <b>mL</b>                                       | <b>milliliter</b>                                                     |
| <b>mm</b>                                       | <b>millimeter</b>                                                     |
| <b>mM</b>                                       | <b>millimolar</b>                                                     |
| <b>MS</b>                                       | <b>mass spectrometry</b>                                              |
| <b>Msl</b>                                      | <b>mean sea level</b>                                                 |
| <b><i>n</i></b>                                 | <b>amount of substance</b>                                            |
| <b>N</b>                                        | <b>nitrogen or normal or number of entities</b>                       |
| <b>N<sub>2</sub></b>                            | <b>nitrogen gas</b>                                                   |
| <b>Na</b>                                       | <b>sodium</b>                                                         |
| <b>NaCl</b>                                     | <b>sodium chloride</b>                                                |
| <b>NaClO<sub>4</sub></b>                        | <b>sodium perchlorate</b>                                             |
| <b>NaOCl</b>                                    | <b>sodium hypochlorite (bleach)</b>                                   |
| <b>NaOH</b>                                     | <b>sodium hydroxide</b>                                               |
| <b>NASA</b>                                     | <b>National Aeronautics and Space Administration</b>                  |
| <b>Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub></b> | <b>sodium thiosulfate</b>                                             |
| <b>Ne</b>                                       | <b>neon</b>                                                           |
| <b>ng</b>                                       | <b>nanogram</b>                                                       |
| <b>NH<sub>4</sub>ClO<sub>4</sub></b>            | <b>ammonium perchlorate</b>                                           |
| <b>NH<sub>4</sub></b>                           | <b>ammonia</b>                                                        |
| <b>Ni</b>                                       | <b>nickel</b>                                                         |
| <b>nL</b>                                       | <b>nanoliter</b>                                                      |
| <b>N<sub>2</sub>O</b>                           | <b>nitrous oxide</b>                                                  |
| <b>NO<sub>2</sub><sup>-</sup></b>               | <b>nitrite</b>                                                        |
| <b>NO<sub>3</sub><sup>-</sup></b>               | <b>nitrate</b>                                                        |
| <b>NO<sub>3</sub>-N</b>                         | <b>nitrate as nitrogen</b>                                            |
| <b>NRC</b>                                      | <b>National Research Council of the National Academies of Science</b> |
| <b>O</b>                                        | <b>oxygen</b>                                                         |

|                               |                                                          |
|-------------------------------|----------------------------------------------------------|
| <sup>16</sup> O               | oxygen-16                                                |
| <sup>17</sup> O               | oxygen-17                                                |
| <sup>18</sup> O               | oxygen-18                                                |
| O <sub>2</sub>                | oxygen gas                                               |
| O <sub>3</sub>                | ozone                                                    |
| OCl <sup>-</sup>              | 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 <sub>4</sub> <sup>3-</sup> | 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 <sub>2</sub>               | sulfur dioxide                                           |
| SO <sub>4</sub> <sup>2-</sup> | 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 ( $\text{ClO}_4^-$ ) has both synthetic and natural sources. Stable isotope analysis of O ( $\delta^{18}\text{O}$ ,  $\delta^{17}\text{O}$ ) and stable ( $\delta^{37}\text{Cl}$ ) and radioactive ( $^{36}\text{Cl}$ ) isotope analysis of Cl in  $\text{ClO}_4^-$  allows natural and synthetic  $\text{ClO}_4^-$  to be distinguished from each other and provides information on the potential origin(s) of natural  $\text{ClO}_4^-$ . The goal of this research effort was to develop an improved understanding of (1) the distribution and isotopic characteristics of natural  $\text{ClO}_4^-$  worldwide, (2) the mechanisms of natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ . The first tasks were designed to improve our understanding of  $\text{ClO}_4^-$  distribution, significant environmental  $\text{ClO}_4^-$  production mechanisms and the stable isotopic signature of the produced  $\text{ClO}_4^-$ . These tasks included the following: (1) determination of the isotopic signature of  $\text{ClO}_4^-$  produced by  $\text{O}_3$  and UV oxidation of  $\text{Cl}^-$  and relevant  $\text{ClO}_x$  species; (2) development of an improved understanding of the occurrence and isotopic signature of natural  $\text{ClO}_4^-$  and its relationship with  $\text{NO}_3^-$  and other anions in arid and semi-arid environments worldwide; and (3) evaluation of the potential for microbial production of  $\text{ClO}_4^-$ . A second set of tasks were designed to better understand plant accumulation of environmental  $\text{ClO}_4^-$ , whether its isotopic signature is altered during uptake and accumulation, and/or whether plants can actually generate  $\text{ClO}_4^-$  via an  $\text{O}_3$ -mediated mechanism.

### ***Results***

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

$\text{ClO}_4^-$  and its relationship with common co-occurring anions such as  $\text{Cl}^-$  and  $\text{NO}_3^-$  among others. The data indicate that  $\text{ClO}_4^-$  is globally distributed in soil and groundwater in arid and semi-arid regions on Earth at concentrations ranging from  $10^{-1}$  to  $10^6$   $\mu\text{g/kg}$ . Generally, the  $\text{ClO}_4^-$  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,  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  co-occur at consistent ratios ( $\text{NO}_3^-/\text{ClO}_4^-$ ) that vary between  $\sim 10^4$  and  $\sim 10^5$ . This is not the case for  $\text{Cl}^-/\text{ClO}_4^-$  ratios, which vary widely among locations. The  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  in arid and semi-arid locations, and associated variations in the isotopic composition of the  $\text{NO}_3^-$ , are consistent with a conceptual model of atmospheric origin, global co-deposition, and variable alteration of the  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  compared to  $\text{Cl}^-$  or  $\text{NO}_3^-$ , accompanied by unique  $\text{ClO}_4^-$  isotopic characteristics, may reflect an unusually efficient, but yet unknown, *in situ* production mechanism, regionally elevated atmospheric  $\text{ClO}_4^-$  production rates, or higher  $\text{ClO}_4^-$  production rates in pre-Pleistocene times.

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

Several potential biological mechanisms of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  samples. Bacterial production of  $\text{ClO}_4^-$  was assessed using (1) various nitrifying cultures and enrichments that oxidize  $\text{NH}_4$  to  $\text{NO}_3^-$ ; (2) natural haloperoxidase enzymes that are known to oxidize  $\text{Cl}^-$  to hypochlorous acid ( $\text{HClO}$ ) and potentially to  $\text{ClO}_4^-$  (possibly via additional photochemical or biological reactions); and (3) organisms capable of oxidizing sulfite or phosphite. A variety of experiments were conducted with  $\text{Cl}^-$  or  $\text{ClO}_x$  precursors in the presence of different enzymes, organisms, and conditions as summarized above. While some  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  production. The other organisms and processes evaluated did not result in  $\text{ClO}_4^-$  formation.

A variety of plant species were also evaluated for their potential to accumulate and even generate  $\text{ClO}_4^-$  via  $\text{O}_3$ -mediated processes. Plants, particularly in arid environments, may contain abundant  $\text{Cl}^-$  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  $\text{ClO}_4^-$ . These factors, the ubiquitous distribution of plants, and the post-industrial increase in  $\text{O}_3$  exposure are consistent with the possibility that tropospheric  $\text{O}_3$  may induce biosynthesis of  $\text{ClO}_4^-$  from  $\text{Cl}^-$  in plants. This was evaluated as a potentially novel source of  $\text{ClO}_4^-$  in the environment.

A broad range of crop species was observed to accumulate  $\text{ClO}_4^-$  from growth media, and these species differed widely in their bioconcentration of the anion. Foliar  $\text{ClO}_4^-$  concentration was greatest in older leaves, which ultimately contribute to the litter layer, suggesting that scavenging of  $\text{ClO}_4^-$  from deeper soil horizons could lead to redistribution on the soil surface. However, there was no evidence that exposure of leaves to ambient  $\text{O}_3$  or at significantly elevated  $\text{O}_3$  induced any increase in tissue contents of  $\text{ClO}_4^-$ . The results indicate that  $\text{O}_3$  does not lead to increased phyto-accumulation or plant biosynthesis of  $\text{ClO}_4^-$ .

The impact of plant accumulation of  $\text{ClO}_4^-$  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  $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ , or  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  between the growth solutions and leaf extracts. In contrast to  $\text{ClO}_4^-$ ,  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  in plant tissue was fractionated substantially (~10-20‰). The  $\epsilon^{15}\text{N}/\epsilon^{18}\text{O}$  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  $\text{ClO}_4^-$  similarly to  $\text{NO}_3^-$ .

Similar to hydroponically grown plants, field grown plants exposed to environmentally relevant  $\text{ClO}_4^-$  concentrations also did not appear to affect foliar  $\text{ClO}_4^-$  isotopic composition.  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  stable isotopes. The spinach had an isotopic composition similar to that of indigenous natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  (i.e., synthetic, Atacama, indigenous) in commercial produce and in other plant-based food products through



stable isotope analysis of plant accumulated  $\text{ClO}_4^-$ . This finding is important because most of the exposure to  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in the environment. Significant progress was made concerning potential mechanisms of its formation, isotopic characterization of natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  can be differentiated by stable isotope methods, and suggest for the first time that the source(s) of  $\text{ClO}_4^-$  in food crops may be determined by isotopic analysis of  $\text{ClO}_4^-$  in plant tissue.

## 1.0 PROJECT BACKGROUND AND OBJECTIVES

### 1.1 Background

In the United States (U.S.), perchlorate ( $\text{ClO}_4^-$ ) 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 ( $\text{OCl}^-$ ) and chlorate ( $\text{ClO}_3^-$ ), are major contributors to the  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ . 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  $\text{ClO}_4^-$  contaminated waters like these for irrigation purposes, vegetation has also been found to contain the  $\text{ClO}_4^-$  anion and could thus be transferred to humans or animals that ingest these crops (Hogue, 2003; Jackson et al., 2005). Along with edible vegetation,  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  occurrence is not limited to anthropogenic production or use. The oldest, most widely known natural source of  $\text{ClO}_4^-$  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 ( $\text{NO}_3^-$ ) salts, which also contain  $\text{ClO}_4^-$  (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,  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  exposure to sensitive populations, such as those described above (National Academies, 2005). The Environmental Protection Agency (EPA) subsequently established a reference dose (RfD) for  $\text{ClO}_4^-$  of  $0.7\mu\text{g}$  per kg body weight per day (USEPA, 2005). Although EPA has not officially set a national drinking water regulation for  $\text{ClO}_4^-$ , 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  $\text{ClO}_4^-$  (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  $\text{ClO}_4^-$  of 2 parts per billion (ppb;  $\mu\text{g/L}$ ) and 6  $\mu\text{g/L}$ , respectively. Other states have followed suit by setting guidance levels for  $\text{ClO}_4^-$  of their own in both drinking water and groundwater ranging from 1 to 18  $\mu\text{g/L}$  (GAO, 2010). Federal regulatory guidelines for  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  provide insight into its ubiquity and its potential for remediation. The  $\text{ClO}_4^-$  anion has a tetrahedral shape, with a central chlorine atom surrounded by four oxygen atoms. The chlorine atom in the  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  present in the southwest US was isotopically distinct from that found in the Atacama Desert, and that natural  $\text{ClO}_4^-$  from both areas was distinct from synthetic  $\text{ClO}_4^-$ . These data were obtained mainly via ESTCP Project ER-200509, whose primary objective was the development and validation of stable isotope methods for chlorine ( $^{37}\text{Cl}$  and  $^{35}\text{Cl}$ ) and oxygen ( $^{18}\text{O}$ ,  $^{17}\text{O}$ , and  $^{16}\text{O}$ ) in  $\text{ClO}_4^-$  (Böhlke et al., 2005, 2009; Sturchio et al., 2006, 2011, 2014; Hatzinger et al., 2011, 2013). Characterization of radioactive  $^{36}\text{Cl}$  in  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$

(Atacama and U.S. indigenous) were more clearly defined and compared to synthetic sources. The isotopic characteristics of these different types of  $\text{ClO}_4^-$  are illustrated in Figure 1.1.1 and 1.1.2 and summarized as follows (from Hatzinger et al., 2013):

- Synthetic  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ . Reported  $\delta^{18}\text{O}$  values of Atacama  $\text{ClO}_4^-$  (-24.8 ‰ to -4.2 ‰) exhibit substantial overlap with the  $\delta^{18}\text{O}$  values of synthetic  $\text{ClO}_4^-$ , but the Atacama  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  has higher  $^{36}\text{Cl}/\text{Cl}$  ratios ( $22 \times 10^{-15}$  to  $590 \times 10^{-15}$ ) compared to synthetic  $\text{ClO}_4^-$ .
- Natural  $\text{ClO}_4^-$  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 Lower 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  $\text{ClO}_4^-$  in the western US (represented by samples from the SHP, MRGB, and LUB) is distinguishable from both Chilean  $\text{ClO}_4^-$  and synthetic  $\text{ClO}_4^-$  when all relevant stable isotope ratios are considered.
- Natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ . 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  $\text{ClO}_4^-$  when all relevant isotope ratios are considered.

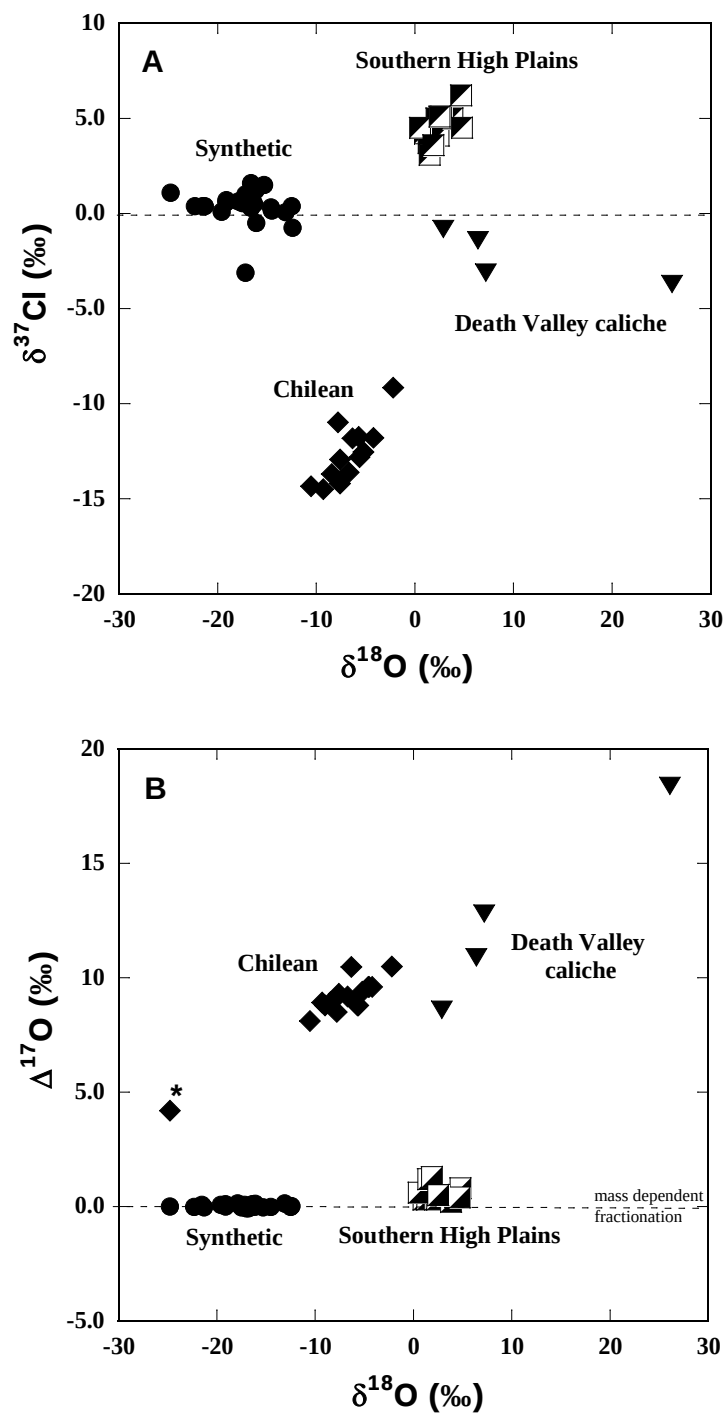


**Figure 1.1.1. Comparison of  $\delta^{37}\text{Cl}$  versus  $\delta^{18}\text{O}$  (Plot A) and  $\Delta^{17}\text{O}$  versus  $\delta^{18}\text{O}$  (Plot B) in natural indigenous  $\text{ClO}_4^-$  in the U.S., natural Chilean  $\text{ClO}_4^-$ , and synthetic  $\text{ClO}_4^-$ . The single value on Plot B labeled with an asterisk is from Bao and Gu (2004). The graphs are from Hatzinger et al. (2013).**

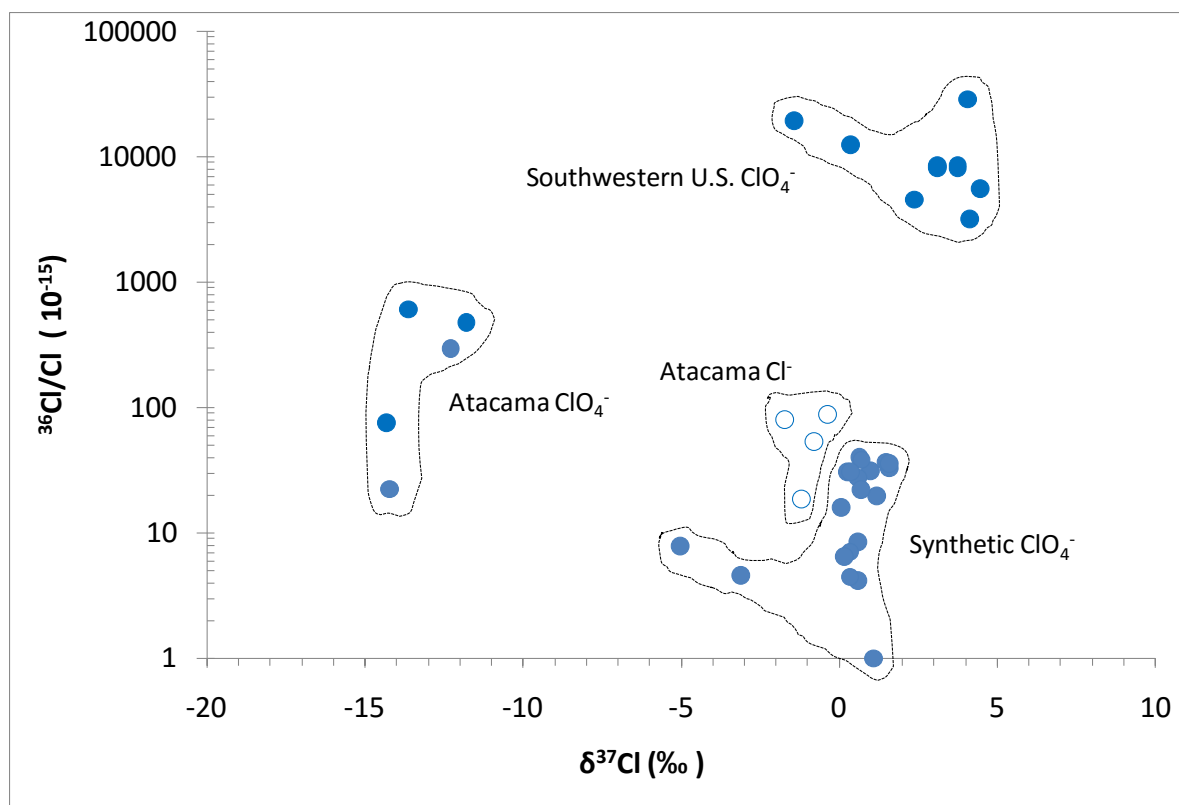


**Figure 1.1.2. Values of  $^{36}\text{Cl}/\text{Cl}$  (mole fraction) versus  $\delta^{37}\text{Cl}$  in representative samples of synthetic  $\text{ClO}_4^-$  reagents and products, natural  $\text{ClO}_4^-$  and  $\text{Cl}^-$  extracted from soil and groundwater from the Atacama Desert, Chile, and 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  $\text{ClO}_4^-$  in the western U.S., and the differing isotopic characteristics between natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  worldwide, mechanisms involved in the natural generation of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  production pathways exist and/or that post-depositional exchanges occur in nature.

Although  $\text{ClO}_4^-$  production pathways for  $\text{O}_3$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  generation, which could help explain the isotopic variation in some of the natural  $\text{ClO}_4^-$  samples. Furthermore, because there is a high potential for plants to uptake, accumulate, and possibly produce  $\text{ClO}_4^-$ , there is a need to examine  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  isotopic compositions or if  $\text{ClO}_4^-$  isotopic compositions vary significantly between species. In addition, little research has examined the ability for plant uptake and transformation processes to fractionate  $\text{ClO}_4^-$ .

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  $\text{ClO}_4^-$  distribution, significant environmental  $\text{ClO}_4^-$  production mechanisms and the stable isotopic signature of the produced  $\text{ClO}_4^-$ .**

***Task 1. Occurrence and isotopic signature of natural  $\text{ClO}_4^-$ .***

At the initiation of this project, there were relatively few data concerning the stable isotope signatures of natural  $\text{ClO}_4^-$ , 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  $\text{ClO}_4^-$  and relationship with  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  and other co-occurring anions, including  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{ClO}_3^-$  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  $\text{ClO}_4^-$  produced by  $\text{O}_3$  and UV-driven oxidation of relevant Cl species.***

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

The isotopic composition of each product and initial reactant (e.g.  $\text{O}_3$ ,  $\text{H}_2\text{O}$ ,  $\text{Cl}$ ,  $\text{OCl}^-$ ,  $\text{ClO}_2^-$ ,  $\text{ClO}_3^-$ , and  $\text{ClO}_4^-$ ) in studies of UV and  $\text{O}_3$  generation of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  consistent with isotopic signatures of reactants and products as well as new constraints on the likely formation mechanisms of natural  $\text{ClO}_4^-$ .



**Task 4. Evaluate the potential for microbial production of  $\text{ClO}_4^-$ .**

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

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

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

Recent reports indicate that a substantial percentage of total exposure may be linked to ingestion of food, mainly leafy vegetables, containing  $\text{ClO}_4^-$  (e.g., USFDA, 2008; Blount et al., 2007). The  $\text{ClO}_4^-$  concentrations in plant leaves and other tissues may reflect a variety of factors, including climatic conditions and species-specific physiological parameters. Sensitivity to ozone ( $\text{O}_3$ ) may be particularly important in determining  $\text{ClO}_4^-$  levels in plants. Tropospheric  $\text{O}_3$  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  $\mu\text{g/L}$  with episodes that may exceed 100  $\mu\text{g/L}$  (Patterson et al., 2000). Ozone and associated reactive oxygen species react with all plant surfaces, but most of the  $\text{O}_3$  damage is caused after these species enter the stomata (Heber et al., 1995). Upon passing the stomata,  $\text{O}_3$  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  $\text{O}_3$  than others. When plant oxidative defense capabilities are overwhelmed, visible foliar damage such as bronzing, flecking, and chlorosis can occur.

As natural  $\text{ClO}_4^-$  could be produced from surface oxidation reactions of  $\text{Cl}^-$  or oxychlorine species with  $\text{O}_3$  (Kang et al., 2008), it is possible that  $\text{ClO}_4^-$  may be directly produced in plant material from interaction of ambient  $\text{O}_3$  with  $\text{Cl}^-$ , especially in certain  $\text{O}_3$  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  $\text{ClO}_4^-$  even in arid climates. The isotopic composition of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  pool by acting as platforms for oxidation of  $\text{Cl}^-$  by  $\text{O}_3$ ;
2. If plants can alter the isotopic signature of  $\text{ClO}_4^-$  during uptake or internal processing;

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

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

A primary objective of this task was to investigate the potential for plants to alter the stable isotopic composition of  $\text{ClO}_4^-$  either during uptake or due to transformation or exchange within the plant. This was accomplished by comparing the stable isotopic compositions of  $\text{ClO}_4^-$  accumulated in hydroponically grown snap bean plants (*Phaseolus vulgaris* L.) with those of the starting reference materials and growth solutions. Results for  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  in these crops and whether sources (e.g., synthetic vs Atacama) could be distinguished from plant-derived  $\text{ClO}_4^-$ . Finally, a commercially obtained spinach sample that was conventionally grown was extracted and the  $\text{ClO}_4^-$  obtained was analyzed for Cl and O isotopes. These isotope data were compared to those from potential  $\text{ClO}_4^-$  perchlorate sources.

## 2.0 PROJECT TASKS

### 2.1 Occurrence and Isotopic Signature of Natural $\text{ClO}_4^-$ 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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  as a minor constituent; and 3) natural  $\text{NO}_3^-$ -rich caliche salt deposits containing  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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”  $\text{ClO}_4^-$  has been described in groundwater beneath a large area (53,000 km<sup>2</sup>) 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<sup>-1</sup>) 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  $\text{ClO}_4^-$ , 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  $\text{ClO}_4^-$  in the diverse environments in which it has been identified.

Stable isotope ratio analysis of Cl and O in  $\text{ClO}_4^-$  has been used to distinguish anthropogenic  $\text{ClO}_4^-$  from Atacama-derived natural  $\text{ClO}_4^-$  in source materials and groundwaters (Bao and Gu, 2004; Böhlke et al., 2005, 2009; Sturchio et al., 2006). Electrochemically produced  $\text{ClO}_4^-$  has relatively well-constrained  $\delta^{37}\text{Cl}$  values (-3 to +2 ‰), but more variable  $\delta^{18}\text{O}$  values (-25 to -13 ‰) likely reflecting variations in the source water and fraction of O lost during production, and  $\Delta^{17}\text{O}$  values that are consistent with mass-dependent fractionation of O isotopes ( $\Delta^{17}\text{O} = 0.0 \pm 0.1$  ‰) (Bao and Gu, 2004; Böhlke et al., 2005, 2009; Sturchio et al., 2006). In contrast, Atacama  $\text{ClO}_4^-$  from saline caliche deposits and imported Chilean nitrate fertilizers has isotopic compositions ( $\delta^{37}\text{Cl} = -15$  to  $-12$  ‰;  $\delta^{18}\text{O} = -25$  to  $-3$  ‰ and  $\Delta^{17}\text{O} = +4$  to  $+11$  ‰) that are distinct from those of synthetic perchlorate. Elevated  $\Delta^{17}\text{O}$  values in  $\text{ClO}_4^-$  from the Atacama Desert have been interpreted as evidence that  $\text{ClO}_4^-$  were formed in part by reactions involving

ozone ( $O_3$ ) in the atmosphere, as atmospheric  $O_3$  is known to be  $^{17}O$ -enriched with measured and modeled  $\Delta^{17}O$  values of  $\sim +30$  to  $40\text{‰}$  (Bao and Gu, 2004; Johnson et al., 2000). Moreover, concentrations of cosmogenic  $^{36}Cl$  are consistent with an upper atmospheric origin for natural  $ClO_4^-$  (Sturchio et al., 2009). Reported kinetic isotope effects accompanying biological reduction of  $ClO_4^-$  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  $ClO_4^-$  when all relevant isotope ratios are considered (i.e.,  $\delta^{37}Cl$ ,  $\delta^{18}O$ , and  $\Delta^{17}O$ ).

At the initiation of this project, almost no information was available regarding the stable isotopic composition of natural  $ClO_4^-$  indigenous to the U.S. Two samples from groundwater wells in the SHP had reported isotopic compositions ( $\delta^{37}Cl = +6.2, +5.1\text{‰}$ ;  $\delta^{18}O = +4.7, +2.5\text{‰}$ ;  $\Delta^{17}O = +0.4, +0.5\text{‰}$ ) that were different from those of both anthropogenic and Atacama  $ClO_4^-$  (Sturchio et al., 2006). These data indicated that the SHP groundwater  $ClO_4^-$  was either a mixture of biologically fractionated, electrochemically produced  $ClO_4^-$  and a much smaller amount of Atacama-like  $ClO_4^-$ , or that it represented an isotopically distinct type of natural  $ClO_4^-$ . As a result of recent studies on the distribution and potential sources of  $ClO_4^-$  in the SHP (Rajagopalan et al., 2006), and rapidly increasing detection of trace  $ClO_4^-$  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  $ClO_4^-$  isotope data are required to confirm this hypothesis and expand the database to other indigenous  $ClO_4^-$  occurrences. In addition, because  $ClO_4^-$  and  $NO_3^-$  typically coexist in terrestrial environments, relations between  $NO_3^-$  and  $ClO_4^-$  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  $ClO_4^-$  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  $NO_3^-$ -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  $ClO_4^-$  distribution and isotopic characteristics, including the origin and isotopic composition of co-occurring  $NO_3^-$ . 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  $ClO_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ . Lastly,  $\text{ClO}_4^-$  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  $\text{NO}_3^-$  concentrations, which resemble those in the Atacama Desert (Böhlke et al., 1997; Ericksen et al., 1988; Noble, L.F. 1931; Michalski et al., 2004).

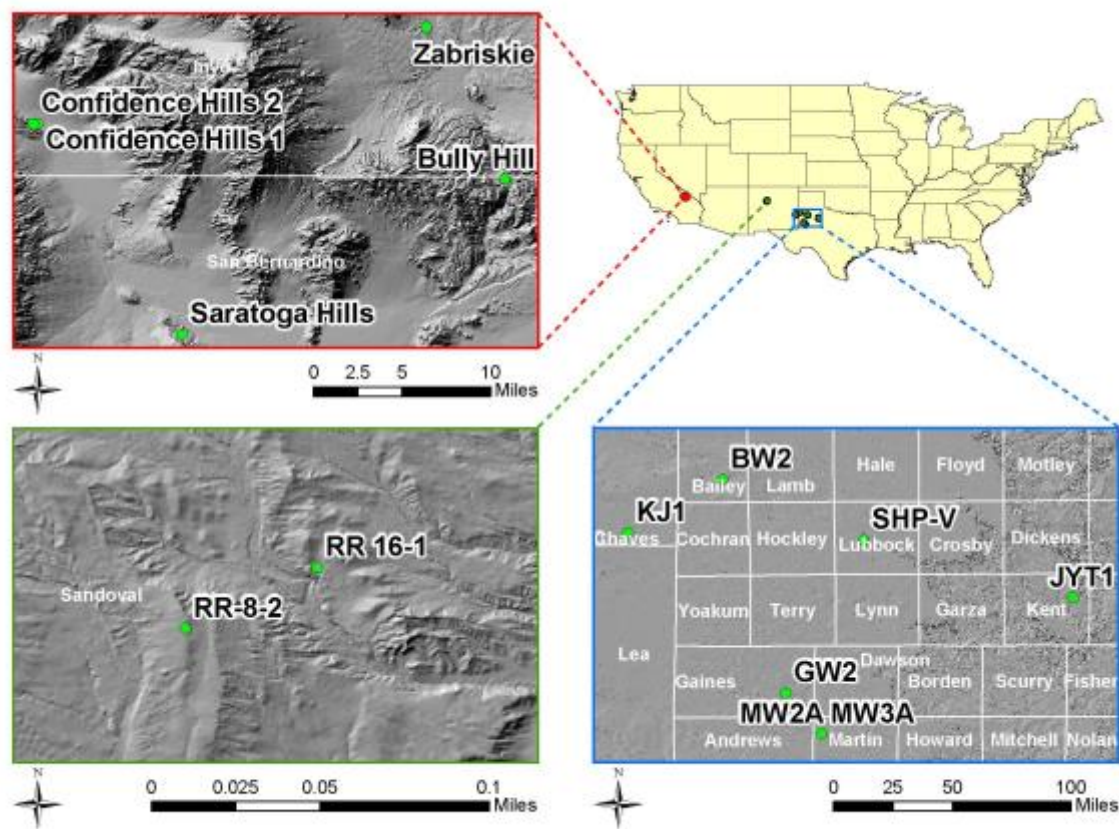


**Figure 2.1.1. Sample locations for natural perchlorate.** Surface deposits were collected from Death Valley, CA (red box), groundwater from the Rio Grande Basin of NM (green box) and vadose soils and groundwater from the SHP of Texas (blue box).

#### 2.1.2.1 Sample Collection

$\text{ClO}_4^-$  in groundwater was collected by pumping water from each well through columns containing  $\text{ClO}_4^-$ -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  $\sim 0.1$  to  $2 \text{ L min}^{-1}$ . 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  $\text{ClO}_4^-$  concentration, with the final objective being to extract at least 5 mg of  $\text{ClO}_4^-$  for purification and isotopic analysis. Groundwater was also collected for major anions, other isotopic analyses ( $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{H}_2\text{O}$ ), major dissolved gases, and environmental tracers including  $^3\text{H}$ ,  $^3\text{He}$ ,  $\text{SF}_6$ , 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  $\text{ClO}_4^-$  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  $\sim 10$  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 ( $\sim 1,000 \text{ cm}^3$ ) packed with Purolite A-530E resin to reduce  $\text{ClO}_4^-$  to  $< 0.05 \mu\text{g L}^{-1}$ . 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- $\mu\text{m}$  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  $\text{ClO}_4^-$  applied to the column and the efficiency of perchlorate removal by the column. A total of  $\sim 5,600 \text{ kg}$  of soil and  $\sim 3,000 \text{ L}$  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  $\text{NO}_3^-$  content of the deposits, which were determined by field testing.  $\text{NO}_3^-$  was used as an indicator of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ -free ( $< 0.05 \mu\text{g L}^{-1}$ ) distilled de-ionized water. The samples were sequentially extracted three times with a  $\sim 1:5$  solid to water mass ratio each time. The  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{Cl}^-$ .

#### 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 ( $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ , organic anions, etc.) and other impurities. Then  $\text{ClO}_4^-$  was eluted with 3-5 BV of a mixed solution of 1M  $\text{FeCl}_3$  and 4M HCl, and is usually concentrated in <0.5 BV of the eluent solution. To remove  $\text{Fe}^{+3}$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  as described by Hatzinger et al. (2011). Finally, the purified and concentrated  $\text{ClO}_4^-$  in solution was crystallized by the addition of CsCl or CsOH to cause supersaturation and precipitation of  $\text{CsClO}_4$ . The  $\text{CsClO}_4$  precipitate was then washed with 90% MeOH and air dried prior to isotopic analysis by isotope-ratio mass spectrometry. Purity of final  $\text{CsClO}_4$  crystals was verified by micro-Raman spectroscopy. Additional details pertaining to the use of A-530E resin for  $\text{ClO}_4^-$  extraction and purification are provided elsewhere (Hatzinger et al., 2011; Gu et al., 2011).

#### 2.1.2.4 Sample Analysis

Purified  $\text{ClO}_4^-$  in the form of  $\text{CsClO}_4$  was shipped to the USGS laboratory in Reston Virginia for analysis of  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  on  $\text{O}_2$  produced by decomposition. Chloride residue from the decomposed  $\text{ClO}_4^-$  was analyzed for  $\delta^{37}\text{Cl}$  at the University of Illinois at Chicago as described below.  $\text{ClO}_4^-$  concentrations were measured by sequential IC-MS/MS with a method detection limit of  $0.05 \mu\text{g L}^{-1}$  (Rao et al., 2007). Major anions ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{Br}^-$ ) were analyzed by ion chromatography following EPA Method 300.0. Major dissolved gases, and stable isotope ratios in  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  ( $\delta^{18}\text{O}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$ ) were analyzed at the USGS in Reston (USGS, 2010).  $\Delta^{17}\text{O}$  analyses of  $\text{NO}_3^-$  were also performed at the USGS in Reston on  $\text{O}_2$  produced by thermal decomposition of purified  $\text{NO}_3^-$  [see next section]. Tritium was analyzed by electrolytic enrichment and scintillation counting at the USGS in Menlo Park, CA.  $^{14}\text{C}$  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  $\delta^{18}\text{O}$ ,  $\Delta^{17}\text{O}$ , and  $\delta^{37}\text{Cl}$  for  $\text{ClO}_4^-$  were determined by off-line sealed-tube decomposition and dual-inlet isotope-ratio mass spectrometry on  $\text{O}_2$  (designated O2-DIIRMS) and  $\text{CH}_3\text{Cl}$ , and the data were calibrated by analyzing  $\text{ClO}_4^-$  reference materials with

the samples as described elsewhere (Hatzinger et al., 2011; Böhlke et al., 2016). Reference values adopted provisionally for USGS37:  $\delta^{37}\text{Cl} = +0.6\text{‰}$ ,  $\delta^{18}\text{O} = -17.0\text{‰}$ , and  $\Delta^{17}\text{O} = 0.0\text{‰}$ ; and for USGS38:  $\delta^{37}\text{Cl} = -87.2\text{‰}$ ,  $\delta^{18}\text{O} = +52.4\text{‰}$ , and  $\Delta^{17}\text{O} = +73.3\text{‰}$ . 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  $\text{ClO}_4^-$  concentrations,  $\delta^{18}\text{O}$  values determined by O2-DIIRMS and CO-CFIRMS methods generally were indistinguishable. For some samples purified from low  $\text{ClO}_4^-$  soils and groundwaters, however, the O2-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  $\text{ClO}_4^-$  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  $\text{NO}_3^-$  were measured by continuous-flow isotope-ratio mass spectrometry on  $\text{N}_2\text{O}$  produced from  $\text{NO}_3^-$  by bacterial reduction (Sigman et al., 2001; Casciotti et al., 2002; Coplen et al., 2004). The data were calibrated by analyzing  $\text{NO}_3^-$  isotope reference materials using calibration data in Böhlke et al. (2003). For USGS34,  $\delta^{15}\text{N} = -1.8\text{‰}$  and  $\delta^{18}\text{O} = -27.9\text{‰}$ ; for USGS32,  $\delta^{15}\text{N} = 180.0\text{‰}$ ; for USGS35,  $\delta^{18}\text{O} = +57.5\text{‰}$ . For samples with elevated  $\Delta^{17}\text{O}$  of  $\text{NO}_3^-$ ,  $\delta^{15}\text{N}$  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  $\text{N}_2\text{O}$  ion ratios, based on the measured  $\Delta^{17}\text{O}$  values of the  $\text{NO}_3^-$  (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  $\Delta^{17}\text{O} = +21\text{‰}$ .

$\Delta^{17}\text{O}$  in  $\text{NO}_3^-$  was measured by dual-inlet isotope-ratio analysis of  $\text{O}_2$  produced by off-line partial decomposition of  $\text{AgNO}_3$  (Michalski et al., 2002).  $\text{NO}_3^-$  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- $\text{KNO}_3$  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  $\text{AgNO}_3$  salt. The  $\text{AgNO}_3$  was heated under vacuum at 520°C while connected to a 5A° mol-sieve trap cooled with liquid  $\text{N}_2$  to collect  $\text{O}_2$ , which was then isolated and transferred to the mass spectrometer and analyzed against tank  $\text{O}_2$ . No adjustments were made to the  $\Delta^{17}\text{O}$  data, as the measured  $\Delta^{17}\text{O}$  values of  $\text{NO}_3^-$  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  $\text{NO}_3^-$ -rich caliche deposits exist in the clay hills around the southern end of Death Valley within the Mojave Desert. These  $\text{NO}_3^-$  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  $\text{NO}_3^-$  concentrations and  $\text{NO}_3^-/\text{Cl}^-$  ratios locally are similar to those of the Atacama  $\text{NO}_3^-$  ores (Böhlke et al., 1997; Ericksen et al., 1988; Noble, 1931; Noble et al., 1922). Isotopic analyses of the Death Valley  $\text{NO}_3^-$  deposits indicate a large fraction of the  $\text{NO}_3^-$  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  $\text{NO}_3^-$  in the Atacama  $\text{NO}_3^-$  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  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  is well established,  $\text{ClO}_4^-$  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,  $\text{ClO}_4^-$  concentrations ranged from 0.25 to 1.7  $\text{mg kg}^{-1}$ , which are the highest reported  $\text{ClO}_4^-$  concentrations in any natural material in North America, but still approximately 1-3 orders of magnitude lower than in the Atacama  $\text{NO}_3^-$ -rich caliches (Ericksen et al., 1981). Interestingly, the  $\text{NO}_3^-$  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 ( $\delta^{18}\text{O}$ ,  $\Delta^{17}\text{O}$ , and  $\delta^{15}\text{N}$ ) 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  $\text{NO}_3^-$  (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  $\delta^{37}\text{Cl}$  values are higher than those of Atacama  $\text{ClO}_4^-$  (including new data reported here) and generally lower than those of synthetic  $\text{ClO}_4^-$ . The  $\delta^{18}\text{O}$  values are higher than those of both Atacama and synthetic  $\text{ClO}_4^-$ . However, the  $\Delta^{17}\text{O}$  values of Death Valley caliche  $\text{ClO}_4^-$  generally are similar to those of Atacama  $\text{ClO}_4^-$  with the exception of the Zabriskie sample ( $\Delta^{17}\text{O} = +18.4\text{‰}$ ), which has the highest  $\Delta^{17}\text{O}$  value reported to date for  $\text{ClO}_4^-$  (Figure 2.1.2). Combined data from Death Valley and Atacama indicate a positive correlation between  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  in caliche  $\text{ClO}_4^-$ , with one end of the correlation line pointing toward the isotopic composition of atmospheric  $\text{O}_3$  and the other end approaching the terrestrial mass-dependent fractionation line at a negative  $\delta^{18}\text{O}$  value (Figure 2.1.3) (see also Bao and Gu, 2004). This pattern is similar to the one defined by  $\text{NO}_3^-$  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

$\text{ClO}_4^-$  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  $\mu\text{g L}^{-1}$  and with no systematic relation between groundwater age and  $\text{ClO}_4^-$  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  $\text{O}_2$ , and major anion concentrations ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{SO}_4^{2-}$ ) that may largely represent atmospheric fluxes with varying amounts of evapotranspiration (Plummer et al., 2004). The  $\delta^{18}\text{O}$  values of the  $\text{NO}_3^-$  in these wells are much lower than those of atmospheric  $\text{NO}_3^-$  and are consistent with values produced by nitrification in soils. The  $\delta^{15}\text{N}$  values of the  $\text{NO}_3^-$  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  $\text{NO}_3^-$  isotopes do not indicate denitrification in the saturated zone, hence it is also unlikely that  $\text{ClO}_4^-$  was reduced in the saturated zone given the residual  $\text{NO}_3^-$  in solution.

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

#### 2.1.3.3 Southern High Plains Groundwater

$\text{ClO}_4^-$  is present in groundwater ( $\sim 0.1$  to 200  $\mu\text{g L}^{-1}$ ) in at least 54 counties covering 155,000  $\text{km}^2$  in the SHP of Texas and New Mexico (Rajagopalan et al., 2004). The distribution and total mass of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $^3\text{H}$ ) to more than 10,000 years (e.g., MW3 with 34 percent modern C and  $^3\text{H}$  near the detection limit); however, there is evidence for mixing of old and young water in some cases (MW2 with low  $^{14}\text{C}$  and post-bomb  $^3\text{H}$ ) (Table 2.1.4).

Samples for  $\text{ClO}_4^-$  isotopic analysis were collected from groundwater at five sites in the SHP, including two previously sampled sites, spread across an area of  $\sim 40,000 \text{ km}^2$  (Figure 2.1.1).  $\text{ClO}_4^-$  concentrations in these samples ranged from 1.8 to  $200 \mu\text{g L}^{-1}$  (Table 2.1.4). The new SHP  $\text{ClO}_4^-$  samples all have similar isotopic compositions that are indistinguishable from those of the two SHP  $\text{ClO}_4^-$  samples analyzed previously (Böhlke et al., 2005) (Figure 2.1.2; Table 2.1.4). Two hypotheses were advanced previously as possible explanations of  $\text{ClO}_4^-$  isotope data in SHP groundwater: (1) it is a mixture of biologically degraded synthetic  $\text{ClO}_4^-$  plus a small ( $\sim 5\%$ ) amount of Atacama  $\text{ClO}_4^-$ ; or (2) it is an isotopically distinct form of natural  $\text{ClO}_4^-$  (Böhlke et al., 2005). Given the reported ubiquity of  $\text{ClO}_4^-$  in the SHP groundwater and soils, its relatively homogeneous isotopic composition in samples with a wide range of concentrations, and its similarity to  $\text{ClO}_4^-$  in old groundwater in the MRGB, it appears likely these data represent a major natural  $\text{ClO}_4^-$  province that is isotopically different from the Atacama and Death Valley caliche  $\text{ClO}_4^-$  occurrences (Figure 2.1.2). The alternative hypothesis (mixing of biodegraded synthetic perchlorate plus Atacama  $\text{ClO}_4^-$ ) would require the unlikely circumstance of a consistent mixing proportion of synthetic and Atacama  $\text{ClO}_4^-$ , along with a constant isotopic shift due to biodegradation, over a wide geographical region.

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

#### 2.1.3.4 Southern High Plains Unsaturated Zone

To further test the hypothesis that  $\text{ClO}_4^-$  in SHP groundwater was remobilized after having accumulated naturally in the unsaturated (vadose) zone, a sample of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  and  $\text{Cl}^-$  concentrations in these accumulations are correlated ( $r=0.59-0.99$ ) and the estimated mass of  $\text{ClO}_4^-$  in the unsaturated zone ( $408 \pm 88 \text{ g ha}^{-1}$ ) is more than sufficient to account for the estimated mass of  $\text{ClO}_4^-$  in SHP groundwater (Rao et al., 2006). The depth profiles of  $\text{ClO}_4^-$  and  $\text{Cl}^-$  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  $\sim 3-4 \text{ m}$  depth and maximum  $\text{Cl}^-$  and  $\text{ClO}_4^-$  concentrations of  $370 \text{ mg kg}^{-1}$  and  $3.3 \mu\text{g kg}^{-1}$ , respectively (Figure 2.1.5). Our profile data are incomplete, as we did not sample below 4 m, yet the mass of  $\text{Cl}^-$  above 4 m represents  $>5,000$  years of accumulation based on a  $\text{Cl}^-$  deposition rate of  $157 \text{ mg/ha-year}$  (Rao et al., 2004). The  $\text{ClO}_4^-$  isotopic composition of this sample ( $\delta^{37}\text{Cl} = +3.7 \text{ ‰}$ ;  $\delta^{18}\text{O} = +2.1 \text{ ‰}$ ; and  $\Delta^{17}\text{O} = +0.8 \text{ ‰}$ ) falls within the range of the SHP and MRGB groundwater samples (Figure 2.1.2). This sample therefore supports the

interpretation that widespread  $\text{ClO}_4^-$  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.**

| Sample ID          | Location                                   | Land Use                                   | Latitude    | Longitude    | Date Sampled | Sample Depth | Sample Description                                          | Elevation (m) |
|--------------------|--------------------------------------------|--------------------------------------------|-------------|--------------|--------------|--------------|-------------------------------------------------------------|---------------|
| Confidence Hills 1 | Death Valley, California, USA (Clay Hills) | National Park                              | N 35 50.350 | W 116 35.256 | 2/16/2006    | <1 m         | Salt-cemented salt and clay                                 | 4             |
| Confidence Hills 2 |                                            |                                            | N 35 50.372 | W 116 35.47  | 1/25/2008    | ~10-30cm     | Salt-cemented green and red clay with chunks of mixed salts | 40            |
| Saratoga Hills     |                                            |                                            | N 35 40.050 | W 116 28.197 |              | ~5-30cm      | Salt-cemented red clay streaked with white veins            | 130           |
| Bully Hill         |                                            |                                            | N 35 47.647 | W 116 12.346 |              | ~5-30cm      | Salt-cemented green and red clay with chunks of mixed salts | 390           |
| Zabriskie          |                                            | Bureau of Land Management                  | N 35 55.103 | W 116 16.234 |              | ~5-30cm      | Salt-cemented green and red clay with chunks of mixed salts | 420           |
| P1                 | Baquedano District, Chile                  | Surface NO <sub>3</sub> <sup>-</sup> mines | S23 11.863  | W69 40.635   | 10/12/2007   | >6m          | Salts in fractured andesite                                 | 1279          |
| P2                 |                                            |                                            | S23 11.863  | W69 40.635   |              | >6m          | Salts in fractured andesite                                 | 1279          |
| P3                 |                                            |                                            | S23 12.059  | W69 40.205   |              | 25cm         | Unconsolidated sediments                                    | 1304          |
| P4                 |                                            |                                            | S23 12.059  | W69 40.205   |              | 50cm         | NaNO <sub>3</sub> caliche                                   | 1304          |
| GJ01               | Chile                                      | Unknown                                    | Unknown     |              | Unknown      | Unknown      | caliche                                                     | Unknown       |
| UIC 24             | Chile                                      | Railway Cut                                | S23.16.53   | W 69.46.14   | 11/03/2007   | 5m           | NO <sub>3</sub> <sup>-</sup> vertical vein in regolith      | 1137          |

**Table 2.1.2. Well information with chemical and isotopic data for groundwater samples.**

|                                                     | Middle Rio Grande Basin |              | Southern High Plains |             |              |               |            |              |
|-----------------------------------------------------|-------------------------|--------------|----------------------|-------------|--------------|---------------|------------|--------------|
| Well Name                                           | RR8                     | RR16         | MW2                  | MW3         | BW2          | JYT1          | GW2        | KJ1          |
| Well Type                                           | PSW <sup>1</sup>        | PSW          | Monitor              | Monitor     | Monitor      | Irrigation    | Monitor    | PSW          |
| County, State                                       | Sandoval, NM            | Sandoval, NM | Martin, TX           | Martin, TX  | Bailey, TX   | Kent, TX      | Gaines, TX | Roosevelt NM |
| Latitude (N)                                        | 35.274                  | 35.292       | 32.417               | 32.416      | 33.966       | 33.246        | 32.667     | 33.643       |
| Longitude (W)                                       | -106.732                | -106.693     | -102.155             | -102.155    | -102.765     | -100.622      | -102.376   | -103.34      |
| Land Surface Elevation (m)                          | 1776                    | 1686         | 881                  | 881         | 1170         | 612           | 954        | 1285         |
| Mid-Screen Elevation (m)                            | 1383                    | 1257         | 843                  | 830         | 1147         | 594           | 943        |              |
| Screen Elevation ± (m)                              | 94                      | 178          | 1.2                  | 3           | 3            | 1.2           | 3          |              |
| Water-Table Elevation (m)                           | 1468                    | 1488         | 846                  | 846         | 1150         | 604           | 945        | 1250         |
| Unsaturated-Zone Thickness (m)                      | 308                     | 198          | 35                   | 35          | 21           | 8             | 9.0        | 35           |
| Sample Date                                         | 9/12/2007               | 9/13/2007    | 11/8/2005            | 11/7/2005   | 11/9/2005    | 1/10/2006     | 6/1/2006   | 3/1/2007     |
| Field parameters                                    |                         |              |                      |             |              |               |            |              |
| T (°C)                                              |                         |              | 22.6                 | 21.5        | 20.4         | 25.9          | 20.1       | 16.1         |
| Specific Conductivity (µs/cm)                       |                         |              | 4709                 | 4508        | 731          | 1818          | 6935       | 2411         |
| Field O <sub>2</sub> (µmol/L)                       |                         |              | 213                  | 174         | 236          | 190           | 91         | 230          |
| Field pH                                            |                         |              | 6.95                 | 8.25        | 7.47         |               | 6.45       | 6.64         |
| Water Chemistry                                     |                         |              |                      |             |              |               |            |              |
| TDS (g/l)                                           |                         |              | 3.06                 | 2.97        | 0.52         | 1.8           | 4.96       | 1.89         |
| F (µmol/L)                                          | 38                      | 58           | 215                  | 192         | 128          |               | 190        | 66           |
| Cl (µmol/L)                                         | 255                     | 598          | 25000                | 37000       | 1600         | 5300          | 29000      | 12000        |
| Br (µmol/L)                                         | 2                       | 2            | 68                   | 111         | 5            | 8             | 98         | 27           |
| NO <sub>3</sub> (µmol/L)                            | 187                     | 137          | 577                  | 209         | 120          | 437           | 129        | 370          |
| SO <sub>4</sub> (µmol/L)                            | 669                     | 1039         | 13000                | 9500        | 1100         | 8100          | 26000      | 7400         |
| ClO <sub>4</sub> (µmol/L)                           | 0.0067                  | 0.0085       | 0.20                 | 0.11        | 0.02         | 0.04          | 2.05       | 0.15         |
| Alkalinity (µmol/L as HCO <sub>3</sub> )            |                         |              | 4200                 | 4000        | 4500         | 3200          | 6600       | 2800         |
| Isotopes                                            |                         |              |                      |             |              |               |            |              |
| H <sub>2</sub> O δ <sup>2</sup> H (‰)               | -85.4                   | -96.8        | -42.6                | -46.6       | -39.0        | -32.1         | -39.0      | -42.8        |
| H <sub>2</sub> O δ <sup>18</sup> O (‰)              | -11.66                  | -12.90       | -5.75                | -6.54       | -5.71        | -5.22         | -5.43      | -5.96        |
| H <sub>2</sub> O <sup>3</sup> H (TU) (±)            | -0.22 (0.27)            | 0.32(0.22)   | 2.54(0.2)            | 0.34(0.17)  | 0.14(0.16)   | 3.68(0.22)    | 1.15(0.19) | 0.15(0.23)   |
| DIC δ <sup>13</sup> C (‰)                           | -7.7                    | -6.4         | -6.4                 | -6.4        | -3.9         | -11.2         | -9.9       | -7.7         |
| DIC <sup>14</sup> C (pmc <sup>2</sup> )(±)          | 11.6(0.2)               | 5.9(0.1)     | 38.4(0.3)            | 33.6(0.3)   | 83.5(0.5)    | 99.7(0.4)     | 98.1(0.4)  | 91.5(0.4)    |
| DIC pmc Age (years) <sup>3</sup>                    | 17300                   | 22700        | 7700                 | 8700        | 1450         | 25            | 151        | 710          |
| SO <sub>4</sub> <sup>2-</sup> δ <sup>34</sup> S (‰) | 1.1(0.2)                | 4.8(0.2)     | 7.3(0.2)             | 7.7(0.2)    | 7.4(0.2)     | 10.2(0.2)     | 8.7(0.2)   | 10.1(0.2)    |
| SO <sub>4</sub> <sup>2-</sup> δ <sup>18</sup> O (‰) | 3.0(0.2)                | 4.5(0.1)     | 7.7(0.1)             | 8.4(0.1)    | 4.6(0.2)     | 8.8(0.2)      | 3.7(0.1)   | 2.9(0.1)     |
| Dissolved Gases                                     |                         |              |                      |             |              |               |            |              |
| CH <sub>4</sub> (µmol/L) (±)                        | 0.000                   | 0.000        | 0.000                | 0.000       | 0.000        | 0.055 (0.007) | 0.000      | 0.000        |
| Ar (µmol/L) (±)                                     | 12.35 (0.02)            | 13. (0.03)   | 13.91 (0.03)         | 13.9 (0.08) | 13.91 (0.09) | 13.52 (0.08)  | 15.22      | 13.21 (0.01) |
| N <sub>2</sub> (µmol/L) (±)                         | 497(2)                  | 534(1)       | 606(2)               | 581(4)      | 557(2)       | 589(8)        | 615        | 547(2)       |
| Ar-N <sub>2</sub> equilibration T (°C)              | 19.2                    | 16.3         | 20.5                 | 18.6        | 15.2         | 23.2          | 12.8       | 18.3         |
| Ar-N <sub>2</sub> excess air (ccSTP/L)              | 1.4                     | 1.7          | 2.5                  | 2.5         | 1.4          | 3.4           | 2.0        | 2.2          |

**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.**

| Site                | Non-soluble fraction | Concentration                 |                    |                                 |                               | ClO <sub>4</sub> <sup>-</sup> isotopes |                                |                                | NO <sub>3</sub> <sup>-</sup> isotopes |                                |                                |
|---------------------|----------------------|-------------------------------|--------------------|---------------------------------|-------------------------------|----------------------------------------|--------------------------------|--------------------------------|---------------------------------------|--------------------------------|--------------------------------|
|                     |                      | ClO <sub>4</sub> <sup>-</sup> | Cl <sup>-</sup>    | NO <sub>3</sub> <sup>-</sup> -N | SO <sub>4</sub> <sup>-2</sup> | δ <sup>37</sup> Cl <sup>d</sup>        | δ <sup>18</sup> O <sup>b</sup> | Δ <sup>17</sup> O <sup>e</sup> | δ <sup>15</sup> N <sup>c</sup>        | δ <sup>18</sup> O <sup>b</sup> | Δ <sup>17</sup> O <sup>e</sup> |
| <b>Death Valley</b> | %                    | mg kg <sup>-1</sup>           | g kg <sup>-1</sup> |                                 |                               | ‰                                      |                                |                                | ‰                                     |                                |                                |
| Confidence Hills 1  | NA                   | 0.25                          | 320                | 1.8                             | 72                            | -0.81                                  | +2.9                           | +8.6                           | +3.4                                  | +25.0                          | +7.9                           |
| Confidence Hills 2  | 49                   | 0.85                          | 180                | 5.5                             | 100                           | -3.1                                   | +7.2                           | +12.8                          | -1.0                                  | +34.5                          | +12.7                          |
| Saratoga Hills      | 78                   | 0.95                          | 63                 | 5.9                             | 23                            | -1.4                                   | +6.4                           | +10.9                          | -0.1                                  | +43.6                          | +15.5                          |
| Bully Hill          | 62                   | 0.82                          | 80                 | 28                              | 6.5                           | NA                                     | NA                             | NA                             | +5.1                                  | +20.9                          | +7.2                           |
| Zabriskie           | 64                   | 1.7                           | 140                | 4.4                             | 39                            | -3.7                                   | +26.1                          | +18.4                          | +3.4                                  | +32.8                          | +10.8                          |
|                     |                      |                               |                    |                                 |                               |                                        |                                |                                |                                       |                                |                                |
| <b>Atacama</b>      |                      |                               |                    |                                 |                               |                                        |                                |                                |                                       |                                |                                |
| P1                  | 42                   | 243                           | 80                 | 12                              | 57                            | -14.3                                  | -10.5                          | +8.1                           | +0.2                                  | +55.5                          | 18.4                           |
| P2                  | 50                   | 328                           | 456                | 44                              | 84                            | -13.6                                  | -6.7                           | +9.2                           | +0.6                                  | +52.8                          | +17.3                          |
| P3                  | 13                   | 113                           | 50                 | 15                              | 51                            | -11.8                                  | -5.7                           | +8.8                           | -2.5                                  | +55.9                          | +20.7                          |
| P4                  | 51                   | 132                           | 61                 | 22                              | 51                            | NA                                     | -7.8                           | +8.5                           | -2.1                                  | +56.5                          | +20.7                          |
| GJ01                | NA                   | 5.4                           | 6                  | 1.2                             | 13                            | -12.5                                  | -5.2                           | +9.4                           | -0.2                                  | +55.3                          | +20.7                          |
| UIC 24 (J-470)      | NA                   | 220                           | 127                | 66                              | 20                            | -12.8                                  | -5.6                           | +8.8                           | +0.4                                  | +49.6                          | +16.2                          |

<sup>a</sup>NA= Not Analyzed; <sup>b</sup>δ<sup>18</sup>O = R<sub>sample</sub>/R<sub>standard</sub>-1, where R= <sup>18</sup>O/<sup>16</sup>O; <sup>c</sup>δ<sup>15</sup>N = R<sub>sample</sub>/R<sub>standard</sub>-1, where R= <sup>15</sup>N/<sup>14</sup>N; <sup>d</sup>δ<sup>37</sup>Cl = R<sub>sample</sub>/R<sub>standard</sub>-1, where R= <sup>37</sup>Cl/<sup>35</sup>Cl; <sup>e</sup>Δ<sup>17</sup>O= [(1+δ<sup>17</sup>O)/(1+ δ<sup>18</sup>O)<sup>0.525</sup>]-1; ‰ = parts per thousand; δ<sup>37</sup>Cl, δ<sup>18</sup>O, and Δ<sup>17</sup>O 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.**

| Well   | Location                                   | ClO <sub>4</sub> <sup>-</sup> | NO <sub>3</sub> <sup>-</sup> -N | Cl <sup>-</sup> | O <sub>2</sub> | <sup>3</sup> H      | Dissolved<br>Inorganic<br>Carbon <sup>14</sup> C | ClO <sub>4</sub> <sup>-</sup><br>δ <sup>37</sup> Cl <sup>d</sup> | ClO <sub>4</sub> <sup>-</sup><br>δ <sup>18</sup> O <sup>b</sup> | ClO <sub>4</sub> <sup>-</sup><br>Δ <sup>17</sup> O <sup>g</sup> | NO <sub>3</sub> <sup>-</sup><br>δ <sup>15</sup> N <sup>c</sup> | NO <sub>3</sub> <sup>-</sup><br>δ <sup>18</sup> O <sup>b</sup> | NO <sub>3</sub> <sup>-</sup><br>Δ <sup>17</sup> O <sup>g</sup> |
|--------|--------------------------------------------|-------------------------------|---------------------------------|-----------------|----------------|---------------------|--------------------------------------------------|------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------|
|        |                                            | μg L <sup>-1</sup>            | mg L <sup>-1</sup>              |                 |                | TU <sup>e</sup> (±) | pmc <sup>f</sup> (±)                             | ‰                                                                |                                                                 |                                                                 | ‰                                                              |                                                                |                                                                |
| MW2A   | SHP,<br>Texas and<br>New<br>Mexico,<br>USA | 24                            | 2.9                             | 1,300           | 6.8            | 2.5 (0.2)           | 38.4 (0.3)                                       | +4.2                                                             | +1.0                                                            | +0.3                                                            | +5.5                                                           | +2.0                                                           | +0.3                                                           |
| MW2B   |                                            |                               |                                 |                 |                |                     |                                                  | +3.7                                                             | +1.4                                                            | +0.3                                                            | NA                                                             | NA                                                             | NA                                                             |
| MW3A   |                                            | 19                            | 8.1                             | 930             | 5.6            | 0.3 (0.2)           | 33.6 (0.3)                                       | +5.0                                                             | +2.2                                                            | +0.3                                                            | +6.6                                                           | +2.0                                                           | NA                                                             |
| MW3B   |                                            |                               |                                 |                 |                |                     |                                                  | +4.1                                                             | +2.4                                                            | +0.3                                                            | NA                                                             | NA                                                             | NA                                                             |
| GW2    |                                            | 200                           | 1.8                             | 1,000           | 2.9            | 1.2 (0.2)           | 98.1 (0.4)                                       | +5.0                                                             | +3.8                                                            | +0.2                                                            | +19.5                                                          | +16.0                                                          | NA                                                             |
| BW2    |                                            | 1.8                           | 1.7                             | 61              | 7.6            | 0.1 (0.2)           | 83.5 (0.5)                                       | +4.5                                                             | +0.55                                                           | +0.6                                                            | +9.1                                                           | +3.7                                                           | NA                                                             |
| JYT1   |                                            | 3.8                           | 6.1                             | 190             | 6.1            | 3.7 (0.2)           | 99.7 (0.4)                                       | +5.1                                                             | +2.7                                                            | +0.5                                                            | +9.9                                                           | +5.2                                                           | NA                                                             |
| KJ1    |                                            | 15                            | 5.2                             | 430             | 7.4            | 0.2 (0.2)           | 91.5 (0.4)                                       | +4.5                                                             | +4.8                                                            | +0.8                                                            | +6.3                                                           | +0.3                                                           | +0.1                                                           |
| RR-8   | MRGB,<br>New<br>Mexico,U<br>SA             | 0.67                          | 2.6                             | 9.0             | NA             | -0.2 (0.3)          | 11.6 (0.2)                                       | +3.1                                                             | +1.5                                                            | +1.2                                                            | +4.8                                                           | -1.2                                                           | NA                                                             |
| RR 16  |                                            | 0.85                          | 1.9                             | 21              | NA             | 0.3 (0.2)           | 5.9 (0.1)                                        | +3.6                                                             | +1.9                                                            | +1.3                                                            | +4.9                                                           | -1.0                                                           | NA                                                             |
| UIC-25 | Chile                                      | 21,600                        | 2,900                           | 7,840           | NA             | NA <sup>a</sup>     | NA                                               | -12.9                                                            | -7.6                                                            | +9.3                                                            | +0.4                                                           | +49.9                                                          | +16.5                                                          |

<sup>a</sup>NA= Not Analyzed; <sup>b</sup>δ<sup>18</sup>O = R<sub>sample</sub>/R<sub>standard</sub>-1, where R= <sup>18</sup>O/<sup>16</sup>O; <sup>c</sup>δ<sup>15</sup>N = R<sub>sample</sub>/R<sub>standard</sub>-1, where R= <sup>15</sup>N/<sup>14</sup>N; <sup>d</sup>δ<sup>37</sup>Cl = R<sub>sample</sub>/R<sub>standard</sub>-1, where R= <sup>37</sup>Cl/<sup>35</sup>Cl;

<sup>e</sup>TU = Tritium Units = 10<sup>18</sup> x <sup>3</sup>H atoms/H atoms. <sup>f</sup>pmc= percent modern carbon (not normalized for δ<sup>13</sup>C); <sup>g</sup>Δ<sup>17</sup>O= [(1+δ<sup>17</sup>O)/(1+ δ<sup>18</sup>O)<sup>0.525</sup>]-1; <sup>h</sup>‰ = parts per thousand; δ<sup>37</sup>Cl, δ<sup>18</sup>O, and Δ<sup>17</sup>O values are referenced to 0 for SMOC, VSMOW, and VSMOW, respectively



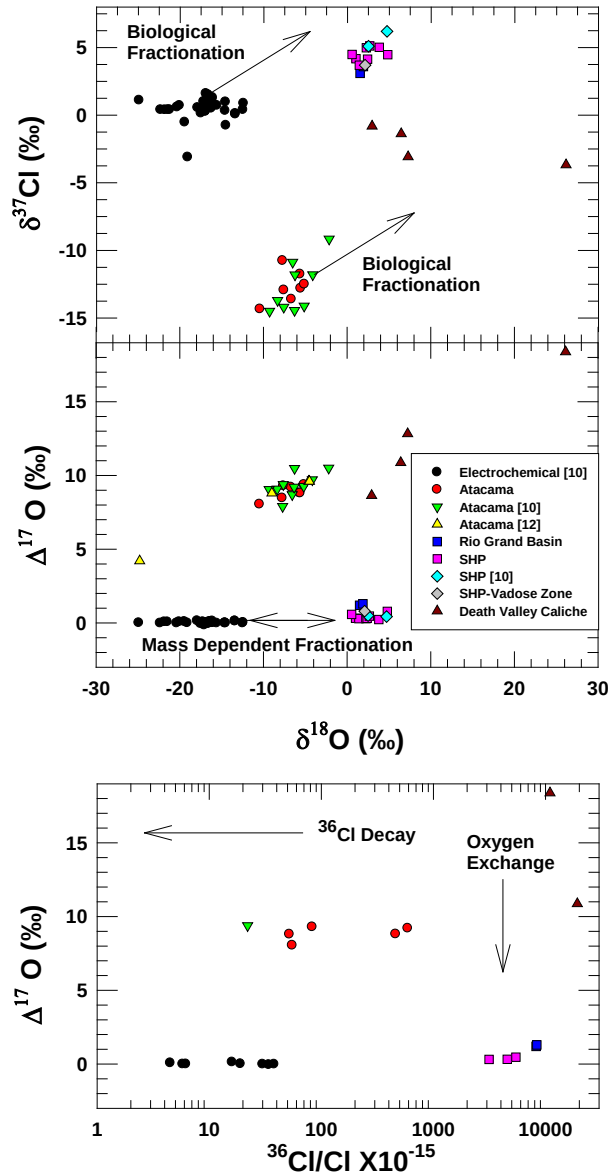


**Figure 2.1.2. Summary of new isotope data for  $\text{ClO}_4^-$  from Southern High Plains (SHP) and Middle Rio Grande Basin (MRGB) groundwater, SHP unsaturated sub-soil, Death Valley and Atacama caliches displayed with previously published  $\text{ClO}_4^-$  isotope data (Böhlke et al., 2005; Sturchio 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  $^{36}\text{Cl}$  decay, and direction due to oxygen exchange.  $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ , and  $\Delta^{17}\text{O}$  values are referenced to 0 for SMOC, VSMOW, and VSMOW, respectively.  $^{36}\text{Cl}$  values are from Sturchio et al., (2009) with the exception of the Zabriskie sample (Death Valley caliche with the highest  $\Delta^{17}\text{O}$ ) (Table 2.1.3).  $\delta^{18}\text{O} = R_{\text{sample}}/R_{\text{standard}} - 1$ , where  $R = ^{18}\text{O}/^{16}\text{O}$ ;  $\delta^{37}\text{Cl} = R_{\text{sample}}/R_{\text{standard}} - 1$ , where  $R = ^{37}\text{Cl}/^{35}\text{Cl}$  and  $\Delta^{17}\text{O} = [(1 + \delta^{17}\text{O})/(1 + \delta^{18}\text{O})^{0.525}] - 1$ .**

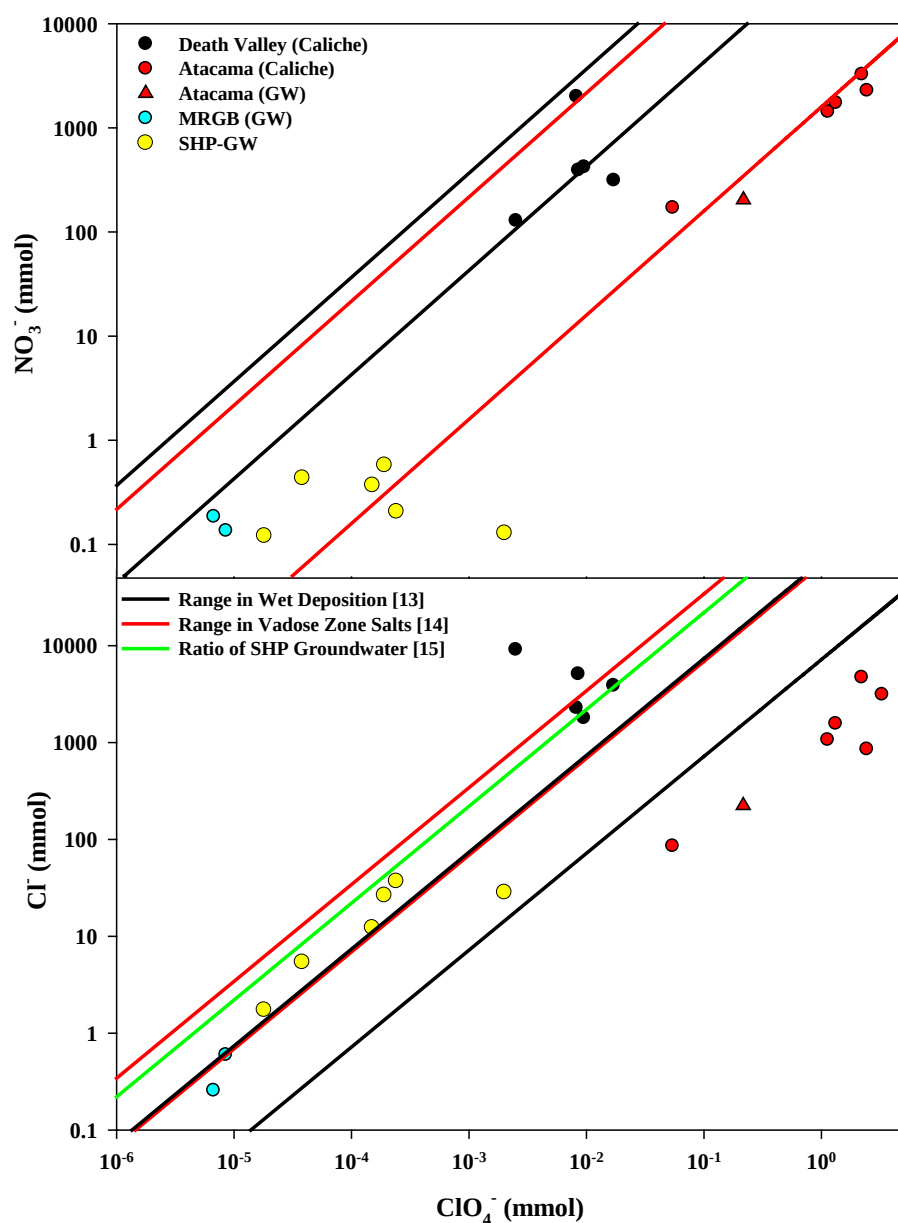


**Figure 2.1.3. Comparison of  $\text{NO}_3^-/\text{ClO}_4^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  molar ratios in new samples with previously published values for wet deposition across the contiguous United States (excluding coastal sites) (Rajagopalan et al., 2009) and vadose-zone salt accumulations in the southwestern United States (Rao et al., 2007). The reported average  $\text{Cl}^-/\text{ClO}_4^-$  ratio is shown for groundwater (GW) in the SHP (Rajagopalan et al., 2006).**

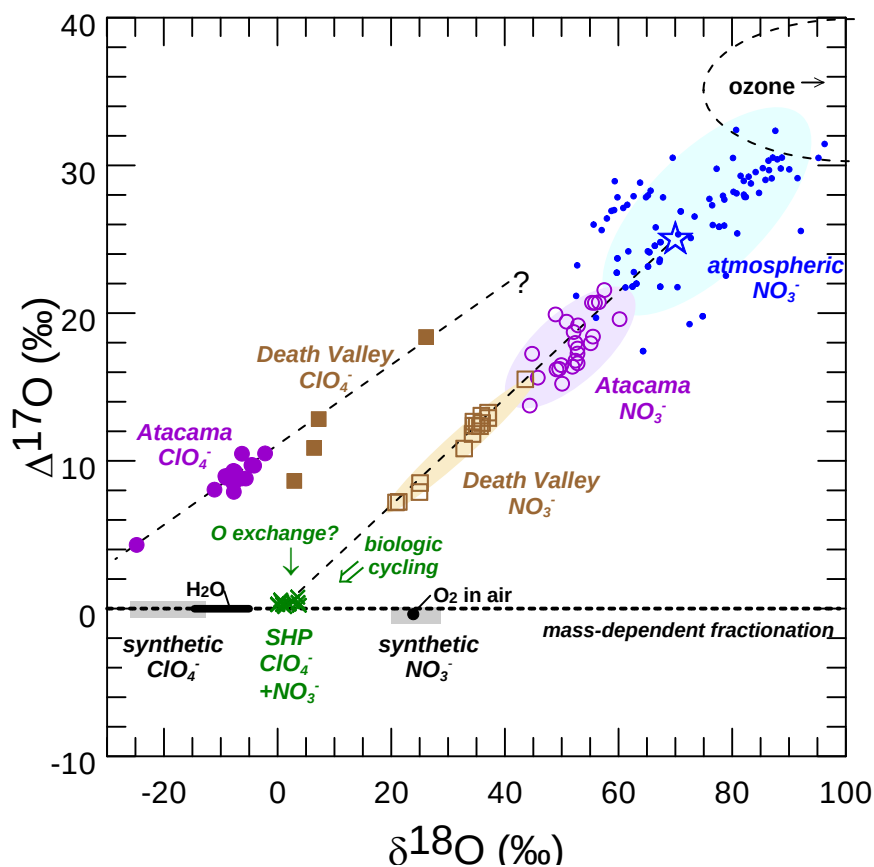


**Figure 2.1.4. Relations between  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  in  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  (data from this study and Böhlke et al., 2005; Sturchio et al., 2006; Bao and Gu, 2004; USGA, 2010; Rao et al., 2010).**  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  values are referenced to 0 for VSMOW (Böhlke et al., 2005). A hypothetical trend line is shown for  $\text{NO}_3^-$  mixtures consisting of biogenic and atmospheric ( $\text{O}_3$ -generated) endmembers. A hypothetical trend line through Atacama and Death Valley  $\text{ClO}_4^-$  could indicate varying expression of  $\text{O}_3$ -generated components.

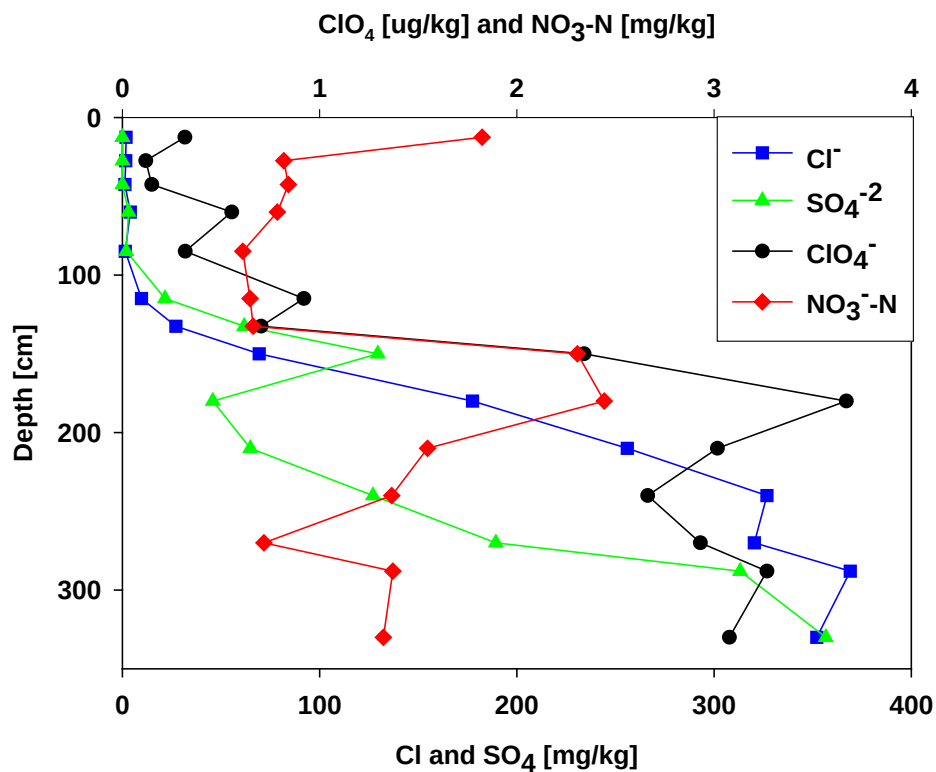


**Figure 2.1.5. Distribution of  $\text{ClO}_4^-$  and other anions with depth below land surface at a rangeland site in the SHP, western Texas.** The water table was more than 15 m below land surface. Concentrations are given as mass of leachable anions per mass of solid material that was leached. Sample 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  $\text{ClO}_4^-$ , based on a combination of  $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ , and  $\Delta^{17}\text{O}$  data. Three major sample groups compared in this study have distinctive isotopic characteristics: Atacama caliche-type  $\text{ClO}_4^-$ , Death Valley caliche-type  $\text{ClO}_4^-$ , and SHP unsaturated-zone and groundwater  $\text{ClO}_4^-$  (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  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  values, including the highest values reported to date for  $\text{ClO}_4^-$  (Figure 2.1.4). Isotopic differences within and among these groups could be variably related to: (1) isotopic compositions of precursor compounds prior to  $\text{ClO}_4^-$  formation, (2) isotopic fractionations accompanying  $\text{ClO}_4^-$  formation, (3) isotopic exchange between  $\text{ClO}_4^-$  and associated chemical species such as  $\text{H}_2\text{O}$  in the environment, and (4) kinetic isotopic fractionation caused by  $\text{ClO}_4^-$ -consuming reactions such as microbial reduction. The importance of these different effects and their relation to regional variation in terrestrial  $\text{ClO}_4^-$  isotopic composition are not completely resolved. Additional constraints are provided by laboratory experiments,  $^{36}\text{Cl}$  concentrations in  $\text{ClO}_4^-$ , and O isotopes in  $\text{NO}_3^-$ .

One possible explanation for isotopic differences between different natural  $\text{ClO}_4^-$  occurrences is different mechanisms and (or) locations of  $\text{ClO}_4^-$  formation. High  $\Delta^{17}\text{O}$  values presumably indicate O transfer from  $\text{O}_3$  during photochemical Cl oxidation in the atmosphere, whereas low  $\Delta^{17}\text{O}$  values could indicate photochemical processes involving oxidants other than  $\text{O}_3$ , possibly occurring in the atmosphere or at the Earth's surface (see Section 2.1). Experiments indicate that  $\text{ClO}_4^-$  can be produced from reactions of oxy-chlorine intermediates ( $\text{HOCl}$ ,  $\text{ClO}_2^-$ ) by irradiation with UV or sunlight (Section 2.1; Kang et al., 2006; Rao et al., 2010). Chlorine dioxide ( $\text{ClO}_2$ ) exists in the stratosphere and may be a precursor compound for a non- $\text{O}_3$ -mediated process of  $\text{ClO}_4^-$  generation (Solomon et al., 1989). The apparent correlation between  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  values in both Atacama and Death Valley  $\text{ClO}_4^-$  could be interpreted as a mixing line between  $\text{O}_3$  mediated and non- $\text{O}_3$ -mediated production mechanisms similar to atmospheric  $\text{NO}_3^-$  (Figure 2.1.4). However, this hypothesis may be difficult to reconcile with the geographic distributions of  $^{36}\text{Cl}/\text{Cl}$  ratios and  $\Delta^{17}\text{O}$  values in  $\text{ClO}_4^-$ . Data for a subset of our samples indicate relatively high concentrations of cosmogenic  $^{36}\text{Cl}$  in natural  $\text{ClO}_4^-$  from both the Death Valley and SHP occurrences, and lower concentrations in Atacama  $\text{ClO}_4^-$  (Figure 2.1.2; Sturchio et al., 2009). High  $^{36}\text{Cl}/\text{Cl}$  ratios in the U.S. samples ( $8,000\text{--}28,000 \times 10^{-15}$ ) seem to preclude formation of  $\text{ClO}_4^-$  from common Cl precursors in the troposphere or near the land surface. The  $^{36}\text{Cl}$  data could be consistent with  $\text{ClO}_4^-$  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  $10^6\text{--}10^7$  years in the Atacama and  $10^4$  years in Death Valley and the SHP) (Sturchio et al., 2009). If the bulk of the  $\text{ClO}_4^-$  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  $\Delta^{17}\text{O}$  values locally among the Death Valley samples, and the near absence of elevated  $\Delta^{17}\text{O}$  values in the other southwestern U.S.  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  isotopic composition could account for local variations in the stable isotopic composition of atmospherically produced  $\text{ClO}_4^-$ , and it could be related to similar processes affecting the isotopic composition of atmospheric  $\text{NO}_3^-$ . Microbial reduction of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  sample groups in Figure 2.1.2 (e.g., microbial reduction follows a specific trajectory in  $\delta^{37}\text{Cl}$  and  $\delta^{18}\text{O}$  and would not alter  $\Delta^{17}\text{O}$  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  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  values in  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  from Death Valley and the Atacama Desert (Figure 2.1.4). Both compounds had relatively high  $\Delta^{17}\text{O}$  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  $\Delta^{17}\text{O}$  values in less arid settings. For  $\text{NO}_3^-$ , although atmospheric production mechanisms have been proposed to vary both spatially and temporally, published analyses indicate consistently high mean annual  $\Delta^{17}\text{O}$  values in atmospheric  $\text{NO}_3^-$  from many regions of the world, and it is considered likely that the full range of  $\Delta^{17}\text{O}$  in terrestrial  $\text{NO}_3^-$  is largely a reflection of varying proportions of atmospheric  $\text{NO}_3^-$  (with high  $\Delta^{17}\text{O}$ ) and biogenic  $\text{NO}_3^-$  (with  $\Delta^{17}\text{O}$  at or near 0) (Michalski et al., 2003, 2004; Ewing et al., 2007). In the current study, the highest  $\Delta^{17}\text{O}$  values in  $\text{NO}_3^-$  (+17 to +21 ‰) were from the Atacama Desert and could indicate 60-80 % of the  $\text{NO}_3^-$  was unaltered atmospheric  $\text{NO}_3^-$ , assuming a long-term average atmospheric  $\text{NO}_3^-$   $\Delta^{17}\text{O}$  value of +25 ‰ (Mcmahon et al., 2006). Somewhat lower  $\Delta^{17}\text{O}$  values in  $\text{NO}_3^-$  from the Death Valley region (+7 to +15 ‰) could indicate somewhat lower fractions of atmospheric  $\text{NO}_3^-$  (30-60 %), based on the same assumption. Much lower  $\text{NO}_3^-$   $\Delta^{17}\text{O}$  values were obtained from two of the SHP samples (+0.1 and +0.3 ‰), indicating almost no unaltered atmospheric  $\text{NO}_3^-$ . From the strong correlation between values of  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  for  $\text{NO}_3^-$  (Figure 2.1.4), we infer that the generally low  $\delta^{18}\text{O}$  values of the other groundwater samples from SHP and MRGB also indicate little or no unaltered atmospheric  $\text{NO}_3^-$  in those samples. The presence or absence of atmospheric isotopic characteristics in  $\text{NO}_3^-$  in these environments is qualitatively consistent with the potential for biological N cycling in local soils (assimilation,  $\text{N}_2$  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  $\text{NO}_3^-$  formerly assimilated into biota and re-mineralized) is a well-documented mechanism for diluting or replacing the atmospheric  $\Delta^{17}\text{O}$  signature of deposited  $\text{NO}_3^-$ , a comparable mechanism for similarly altering the isotopic composition of  $\text{ClO}_4^-$  has not been shown. Therefore, although terrestrial biologic processes can account for O isotopic variations in  $\text{NO}_3^-$ , and although the distribution of  $\Delta^{17}\text{O}$  variations in both  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  appear to be related spatially to local biologic activity, it is not yet possible to attribute  $\text{ClO}_4^-$  isotopic variation to the same processes that generally are thought to affect  $\text{NO}_3^-$  isotopes in these environments.

Another possible explanation for local variations in natural terrestrial  $\text{ClO}_4^-$  derived from the atmosphere is post-deposition isotope exchange (partial equilibration). Isotope exchange between  $\text{ClO}_4^-$  and  $\text{H}_2\text{O}$  would be expected to cause a decrease in  $\Delta^{17}\text{O}$  and could be consistent with the  $\text{ClO}_4^-$  isotope data if exchange was more advanced in wetter or more biologically active environments. Equilibrium O isotope fractionation factors between  $\text{ClO}_4^-$  and  $\text{H}_2\text{O}$  are not known, but we expect  $\delta^{18}\text{O}$  of  $\text{ClO}_4^-$  could be higher than  $\delta^{18}\text{O}$  of coexisting  $\text{H}_2\text{O}$  by analogy with reported fractionation effects ranging from about +14 to +30 ‰ for  $\text{SO}_4^{2-}$ ,  $\text{HSO}_4^-$ ,  $\text{NO}_3^-$ , and  $\text{NO}_2^-$  at room temperature (Mizutani et al., 1969; Zeebe et al., 2010; Böhlke et al., 2003; Casciotti et al., 2007). Therefore, both the  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  values of SHP and MRGB  $\text{ClO}_4^-$  could be qualitatively consistent with partial O isotopic exchange of Death Valley-type  $\text{ClO}_4^-$  with local  $\text{H}_2\text{O}$  ( $\delta^{18}\text{O} = -13$  to  $-5$  ‰) (Figure 2.1.4). Theoretical calculations indicate that  $\delta^{37}\text{Cl}$  of  $\text{ClO}_4^-$  could be 73 ‰ higher than that of coexisting  $\text{Cl}^-$  at equilibrium (Schauble et al., 2003). Thus, the relatively high  $\delta^{37}\text{Cl}$  values of SHP and MRGB  $\text{ClO}_4^-$  might indicate partial exchange of Cl isotopes between  $\text{ClO}_4^-$  and  $\text{Cl}^-$  (or another Cl species) with increasing moisture and(or) biologic activity if the  $\text{ClO}_4^-$  source(s) had relatively low  $\delta^{37}\text{Cl}$ , as in Atacama or Death Valley  $\text{ClO}_4^-$ . 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  $\delta^{37}\text{Cl}$  and  $\Delta^{17}\text{O}$ ). Limited data indicate abiotic exchange of O isotopes between  $\text{ClO}_4^-$  and  $\text{H}_2\text{O}$  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  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  (Böhlke et al., 2003; Blake et al., 1997; Bunton et al., 1953). Field data indicate that Atacama  $\text{ClO}_4^-$  introduced into humid soils and groundwaters in the eastern U.S., and synthetic  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  sources, other than to say that some of the Atacama and Death Valley  $\text{ClO}_4^-$  probably formed as a result of reactions with  $\text{O}_3$ . The data permit the interpretation that natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  sources in the environment. Nevertheless, despite uncertainty about processes responsible for some of the isotopic variations, this study indicates that natural  $\text{ClO}_4^-$  indigenous to the southwestern U.S. is distinguishable from synthetic  $\text{ClO}_4^-$  and from imported Atacama  $\text{ClO}_4^-$  on the basis of isotopic composition. These differences in isotopic composition may find important applications in resolving questions of  $\text{ClO}_4^-$  source apportionment for contaminated water supplies.

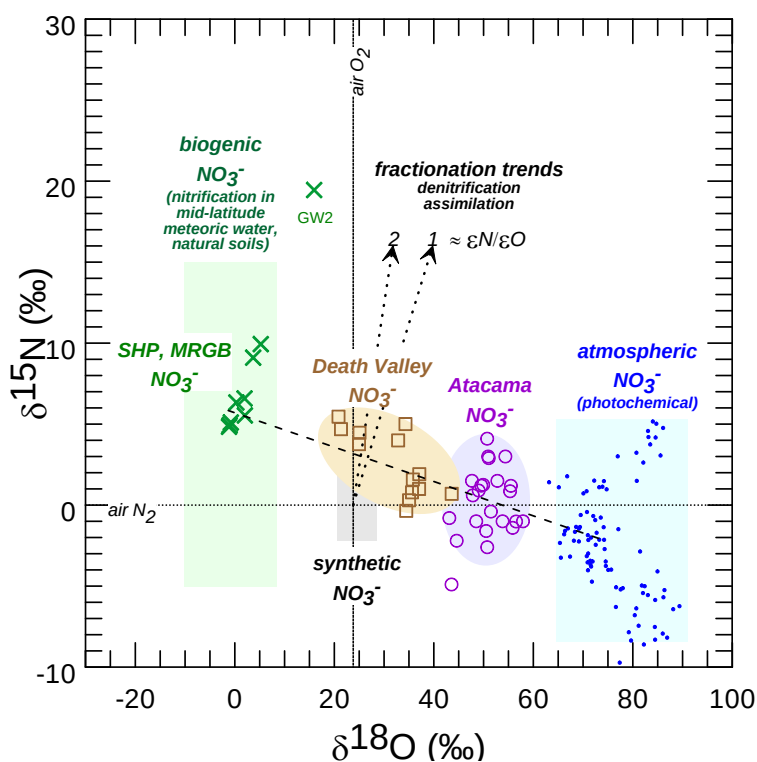


**Figure 2.1.6. Relation between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  in groundwater and leachate samples.** A rough inverse correlation between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  shown here is consistent with varying mixtures of natural biogenic  $\text{NO}_3^-$  and atmospheric  $\text{NO}_3^-$  indicated by a positive correlation between  $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  (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  $\text{ClO}_4^-$  is present at low concentration throughout the water of the Great Lakes. The only previously published report of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in the range of 0.16 to 0.33  $\mu\text{g/L}$ , and concentrations in all samples from the open waters of the Great Lakes were below that study's method detection limit of 0.19  $\mu\text{g/liter}$  (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 Schaetzl, 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  $\text{ClO}_4^-$  present in the Great Lakes is from direct atmospheric deposition, and thus its isotopic composition may be representative of the indigenous atmospheric  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  inventories in the Great Lakes, characterize isotopic compositions of  $\text{ClO}_4^-$  ( $\Delta^{17}\text{O}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{37}\text{Cl}$ ,  $^{36}\text{Cl}/\text{Cl}$ ), and to use these measurements to identify the source(s) of  $\text{ClO}_4^-$ , 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  $\text{ClO}_4^-$  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  $\mu\text{m}$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\mu\text{m}$ ) 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 ( $^{18}\text{O}$ ) labeled  $\text{ClO}_4^-$  internal standard. Sample concentrations were determined from the ratio of the sample

$\text{ClO}_4^-$  peak to the internal standard peak, with a reporting limit of 0.05  $\mu\text{g/L}$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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,  $\text{ClO}_4^-$  was eluted by tetrachloroferrate ( $\text{FeCl}_4^-$ ) in a solution containing 1 mol/L  $\text{FeCl}_3$  and 4 mol/L HCl (Section 2.2; Bao and Gu., 2004; Gu et al., 2001; 2007). The  $\text{ClO}_4^-$ -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 %  $\text{H}_2\text{O}_2$ . An aliquot of this solution was used for ion chromatographic analysis of the amount of  $\text{ClO}_4^-$  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  $\text{Cl}^-$  removal and Ba-treated cation-exchange columns for  $\text{SO}_4^{2-}$  removal. The solution was then passed through solid-phase extraction (SPE) columns for removal of organics, and finally mixed with excess CsOH to precipitate  $\text{CsClO}_4$ . Final purification of perchlorate samples were conducted by precipitation and recrystallization of tetra-n-pentylammonium perchlorate ( $\text{TPAClO}_4$ ) salts (Dosch, 1968). The sample processing blank for perchlorate was about 50  $\mu\text{g}$  (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  $\text{TPAClO}_4$ . 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<sup>+</sup> 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. <sup>34</sup>SH contribution to <sup>35</sup>Cl 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 <sup>16</sup>OH interference on <sup>17</sup>O. This <sup>16</sup>OH interference caused relatively large errors for the <sup>17</sup>O results, therefore SIMS results for <sup>17</sup>O/<sup>16</sup>O 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 <sup>16</sup>O, and an electron multiplier for <sup>17</sup>O and <sup>18</sup>O, with data collection times of 1 sec, 10 sec, and 2 sec per cycle, respectively, for a total of 100 cycles. Chlorine isotopes <sup>35</sup>Cl and <sup>37</sup>Cl 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 KClO<sub>4</sub> isotopic reference materials USGS37 and USGS38, which were converted to TPAClO<sub>4</sub>. Stable isotope ratios are reported in the conventional delta (δ) notation, as follows:

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

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

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 δ<sup>18</sup>O and ±1.0 ‰ for δ<sup>37</sup>Cl. SIMS produced acceptable results for δ<sup>18</sup>O and δ<sup>37</sup>Cl analysis but <sup>17</sup>O/<sup>16</sup>O measurements encountered difficulties in eliminating the effect of large <sup>16</sup>OH interference on <sup>17</sup>O (which may have been related to the use of TPAClO<sub>4</sub> as a target).

#### 2.2.2.5. Isotopic Analysis of Perchlorate by Dual-inlet IRMS

Because of the difficulty encountered in precise determination of <sup>17</sup>O/<sup>16</sup>O ratios by SIMS, samples were converted from TPAClO<sub>4</sub> to KClO<sub>4</sub> by using 0.5 mol/L KOH in absolute ethanol solution, for measurement of oxygen isotope ratios by dual-inlet IRMS of O<sub>2</sub> produced by sealed-tube decomposition of KClO<sub>4</sub> at 600°C. Reported <sup>17</sup>O/<sup>16</sup>O ratios are given in units of Δ<sup>17</sup>O, where:

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

Analytical precision of Δ<sup>17</sup>O based on replicate measurements of USGS-37 KClO<sub>4</sub> isotopic reference material is ±0.2 ‰.

#### 2.2.2.6 $^{36}\text{Cl}$ Abundance Analysis by AMS

Accelerator mass spectrometry (AMS) measurements of  $^{36}\text{Cl}$  isotopic abundances in AgCl (prepared from the KCl produced by decomposition of  $\text{KClO}_4$  for IRMS  $\Delta^{17}\text{O}$  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  $^{36}\text{Cl}$  analyses. The  $^{36}\text{Cl}$  results were corrected for the instrumental background and are reported in units of  $^{36}\text{Cl}/\text{Cl} \times 10^{-15}$  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  $\text{ClO}_4^-$  concentrations in the Great Lakes, and the concentrations ranged from 0.05 to 0.13  $\mu\text{g}/\text{L}$  (averages per lake are provided in Table 2.2.1 and all data are provided in Table 2.2.2). The profiles of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\mu\text{g}/\text{L}$ , consistent with perchlorate removal by the resin columns.

**Table 2.2.1. Concentrations and isotopic compositions of perchlorate from the Great Lakes.**

| <i>Lakes</i>                  | <b>Sample<sup>a</sup><br/>volume (L)</b> | <b>Mean ClO<sub>4</sub><sup>-</sup><br/>(µg/L)<sup>c</sup></b> | <b>δ<sup>37</sup>Cl<br/>(‰)</b> | <b>δ<sup>18</sup>O<br/>(‰)</b> | <b>Δ<sup>17</sup>O<br/>(‰)</b> | <b><sup>36</sup>Cl/Cl<br/>(10<sup>-15</sup>)</b> |
|-------------------------------|------------------------------------------|----------------------------------------------------------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------------------------|
| <b>Superior R<sup>b</sup></b> | 86000                                    | 0.06                                                           | +3.9                            | -4.8                           | +2.7                           | 71200 ± 1700                                     |
| <b>Superior P<sup>b</sup></b> | 73000                                    | 0.06                                                           | +4.0                            | -3.4                           | +2.6                           | 61900 ± 1100                                     |
| <b>Michigan</b>               | 31780                                    | 0.10                                                           | +3.3                            | -1.3                           | +1.7                           | 26600 ± 800                                      |
| <b>Huron</b>                  | 23780                                    | 0.11                                                           | +3.2                            | -2.7                           | +1.6                           | 15800 ± 600                                      |
| <b>Erie</b>                   | 15260                                    | 0.08                                                           | +3.2                            | +4.0                           | +1.8                           | 9300 ± 300                                       |
| <b>Ontario</b>                | 19700                                    | 0.09                                                           | +3.0                            | +1.9                           | +1.7                           | 7400 ± 300                                       |

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

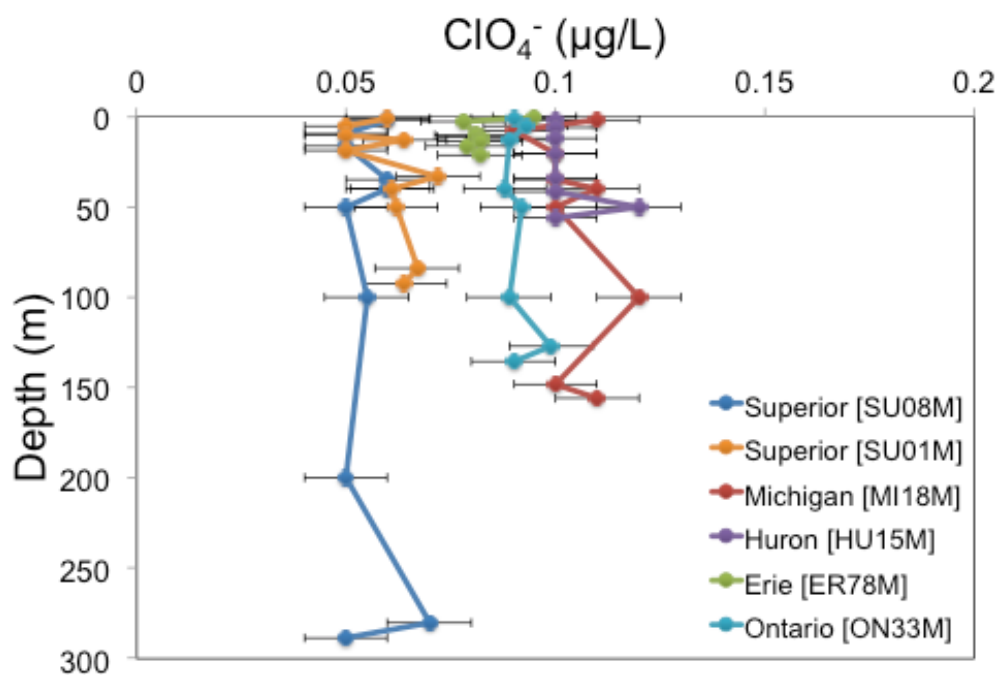


**Figure 2.2.1. Perchlorate concentration vs. depth in the Great Lakes.**

**Table 2.2.2. Perchlorate concentrations in the water samples from the Great Lakes.**

| <b>Lake</b>     | <b>Date</b>  | <b>Station</b> | <b>Depth (m)</b> | <b>ClO<sub>4</sub><sup>-</sup><br/>(µg/L)</b> | <b>Mean ClO<sub>4</sub><sup>-</sup><br/>(µg/L)</b> |
|-----------------|--------------|----------------|------------------|-----------------------------------------------|----------------------------------------------------|
| <b>Superior</b> | August, 2008 | SU01M          | 1.5              | 0.06                                          |                                                    |
|                 | August, 2008 | SU01M          | 5.1              | 0.05                                          |                                                    |
|                 | August, 2008 | SU01M          | 10.5             | 0.05                                          |                                                    |
|                 | August, 2008 | SU01M          | 13.1             | 0.06                                          |                                                    |
|                 | August, 2008 | SU01M          | 18.8             | 0.05                                          |                                                    |
|                 | August, 2008 | SU01M          | 33               | 0.07                                          |                                                    |
|                 | August, 2008 | SU01M          | 40.2             | 0.06                                          |                                                    |
|                 | August, 2008 | SU01M          | 50.1             | 0.06                                          |                                                    |
|                 | August, 2008 | SU01M          | 84.1             | 0.07                                          |                                                    |
|                 | August, 2008 | SU01M          | 92.6             | 0.06                                          |                                                    |
|                 | August, 2008 | SU08M          | 1.6              | 0.06                                          |                                                    |
|                 | August, 2008 | SU08M          | 10               | 0.05                                          |                                                    |
|                 | August, 2008 | SU08M          | 16.1             | 0.05                                          |                                                    |
|                 | August, 2008 | SU08M          | 35               | 0.06                                          |                                                    |
|                 | August, 2008 | SU08M          | 39.9             | 0.06                                          |                                                    |
|                 | August, 2008 | SU08M          | 50               | 0.05                                          |                                                    |
|                 | August, 2008 | SU08M          | 99.9             | 0.06                                          |                                                    |
|                 | August, 2008 | SU08M          | 200              | 0.05                                          |                                                    |
|                 | August, 2008 | SU08M          | 280.4            | 0.07                                          |                                                    |
|                 | August, 2008 | SU08M          | 288.5            | 0.05                                          | 0.06 ± 0.01                                        |
| <b>Michigan</b> | August, 2007 | MI18M          | 1.6              | 0.11                                          |                                                    |
|                 | August, 2007 | MI18M          | 8.3              | 0.09                                          |                                                    |
|                 | August, 2007 | MI18M          | 20.5             | 0.10                                          |                                                    |
|                 | August, 2007 | MI18M          | 35.1             | 0.10                                          |                                                    |
|                 | August, 2007 | MI18M          | 40.3             | 0.11                                          |                                                    |

|              |              |           |                        |      |             |
|--------------|--------------|-----------|------------------------|------|-------------|
|              | August, 2007 | MI18M     | 50                     | 0.10 |             |
|              | August, 2007 | MI18M     | 100                    | 0.12 |             |
|              | August, 2007 | MI18M     | 147.9                  | 0.10 |             |
|              | August, 2007 | MI18M     | 156                    | 0.11 |             |
|              | August, 2007 | MI27M INT | 0                      | 0.10 |             |
|              | August, 2007 | MI11 INT  | 0                      | 0.09 |             |
|              | August, 2007 | MI17 INT  | 0                      | 0.10 |             |
|              | August, 2007 | MI23 INT  | 0                      | 0.10 |             |
|              | August, 2007 | MI19 SURF | Surface                | 0.10 |             |
|              | August, 2007 | MI17 SURF | Surface                | 0.10 |             |
|              | August, 2007 | MI32 SURF | Surface                | 0.10 |             |
|              | August, 2007 | MI34 SURF | Surface                | 0.10 |             |
|              | August, 2008 | MI18M     | Sea Chest <sup>a</sup> | 0.11 |             |
|              | August, 2008 | MI27M     | Sea Chest              | 0.12 |             |
|              | August, 2008 | MI41M     | Sea Chest              | 0.13 | 0.10 ± 0.01 |
| <b>Huron</b> | August, 2007 | HU15M     | 1.7                    | 0.10 |             |
|              | August, 2007 | HU15M     | 6.1                    | 0.10 |             |
|              | August, 2007 | HU15M     | 12                     | 0.10 |             |
|              | August, 2007 | HU15M     | 20.4                   | 0.10 |             |
|              | August, 2007 | HU15M     | 34.2                   | 0.10 |             |
|              | August, 2007 | HU15M     | 42                     | 0.10 |             |
|              | August, 2007 | HU15M     | 50                     | 0.12 |             |
|              | August, 2007 | HU15M     | 56.4                   | 0.10 |             |
|              | August, 2007 | HU32 INT  | 0                      | 0.13 |             |
|              | August, 2007 | HU37 INT  | 0                      | 0.11 |             |
|              | August, 2007 | HU53 INT  | 0                      | 0.12 |             |
|              | August, 2007 | HU38 SURF | Surface                | 0.11 |             |
|              | August, 2007 | HU48 SURF | Surface                | 0.10 |             |



|                |              |           |           |      |             |
|----------------|--------------|-----------|-----------|------|-------------|
|                | August, 2007 | HU37 SURF | Surface   | 0.11 |             |
|                | 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 |
| <b>Erie</b>    | 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 |
| <b>Ontario</b> | 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 |

<sup>a</sup> 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  $\text{ClO}_4^-$  extracted from the Great Lakes provide constraints on possible source(s) and may indicate geochemical processes affecting  $\text{ClO}_4^-$  concentrations in the lakes. Values of  $\delta^{37}\text{Cl}$  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  $\pm 1.0$  ‰. Values of  $\delta^{18}\text{O}$  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  $\Delta^{17}\text{O}$  values ranging from +1.6 ‰ to +2.7 ‰. Lake Superior has a larger  $\Delta^{17}\text{O}$  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.

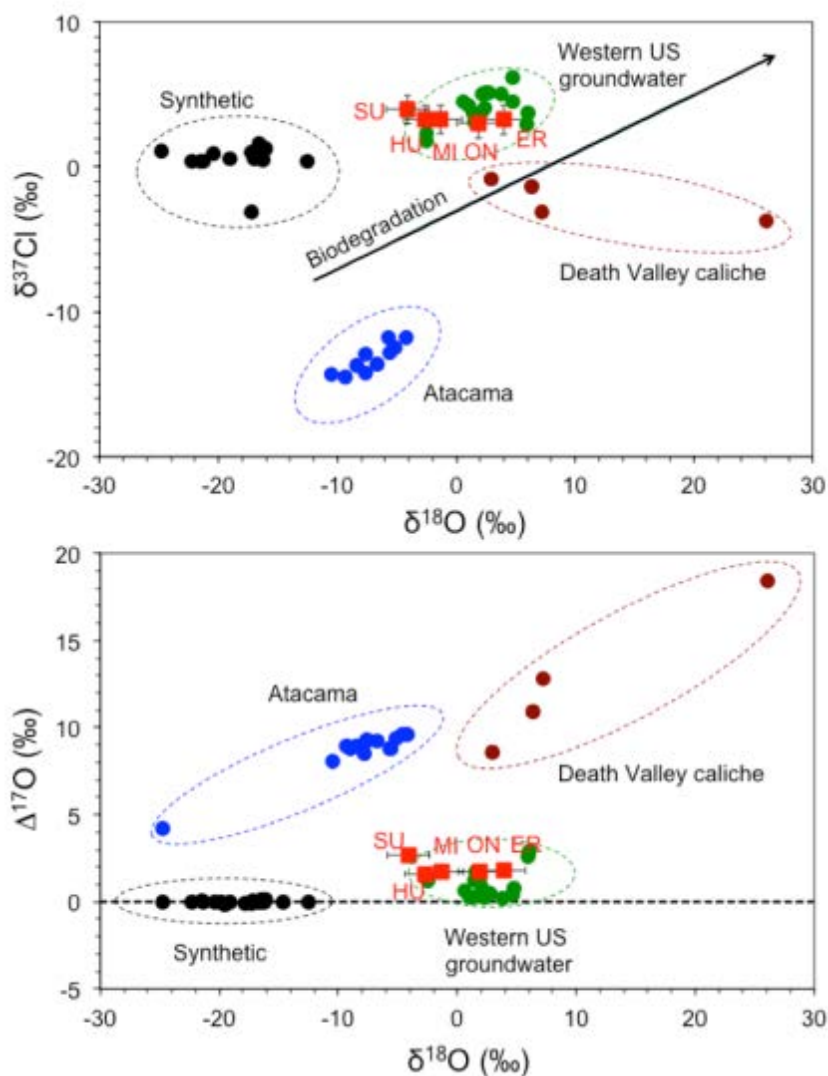


**Figure 2.2.2. Diagrams showing  $\delta^{37}\text{Cl}$  vs.  $\delta^{18}\text{O}$  values (upper diagram) and  $\Delta^{17}\text{O}$  vs.  $\delta^{18}\text{O}$  values (lower diagram) for Great Lakes perchlorate samples.** Published isotopic data from Atacama, Death Valley, western US groundwater 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  $\delta^{18}\text{O}$  values of  $\text{ClO}_4^-$  from the Great Lakes are generally divided into two groups with Lakes Superior, Michigan and Huron having more negative  $\delta^{18}\text{O}$  values (-4.1 ‰ to -1.3 ‰), and Lakes Erie and Ontario having more positive  $\delta^{18}\text{O}$  values (+1.9 ‰ to +4.0 ‰). The spatial variability of the  $\delta^{18}\text{O}$  values of perchlorate in the Great Lakes is similar to the spatial variability of the  $\delta^{18}\text{O}$  values of  $\text{H}_2\text{O}$  (-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 ( $\delta^{18}\text{O}$ ) variability of Great Lakes  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  isotopic compositions. However, the experimentally measured rate of  $\text{ClO}_4^-$ -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  $\text{ClO}_4^-$ -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 ( $^{36}\text{Cl}$ ) is a cosmogenic radionuclide with a half-life of 301,000 years. Atmospheric  $^{36}\text{Cl}$  is primarily produced by cosmic-ray spallation of  $^{40}\text{Ar}$  (Lehmann et al., 1993; Lal et al., 1967). Isotopic abundance of  $^{36}\text{Cl}$  in Great Lakes  $\text{ClO}_4^-$  decreases from west to east, from a high  $^{36}\text{Cl}/\text{Cl}$  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  $^{36}\text{Cl}/\text{Cl}$  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  $\text{ClO}_4^-$  generally resembles that of indigenous natural  $\text{ClO}_4^-$  from the western USA in terms of  $\delta^{37}\text{Cl}$  values and  $^{36}\text{Cl}/\text{Cl}$  ratios (Figure 2.2.3). The isotopic composition of indigenous natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  throughout the Great Lakes (Figures 2.2.2 and 2.2.3). Based on measurements performed to date, all pre-bomb indigenous natural  $\text{ClO}_4^-$  has high  $^{36}\text{Cl}/\text{Cl}$  ratios relative to synthetic and Atacama  $\text{ClO}_4^-$ ; the highest  $^{36}\text{Cl}/\text{Cl}$  ratios were found in indigenous  $\text{ClO}_4^-$  that may have incorporated bomb-pulse  $^{36}\text{Cl}$ .<sup>35</sup> The relatively high  $^{36}\text{Cl}/\text{Cl}$  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  $^{36}\text{Cl}$  by neutron activation of  $^{35}\text{Cl}$  in seawater, and injected this  $^{36}\text{Cl}$  into the stratosphere. The  $^{36}\text{Cl}$  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.

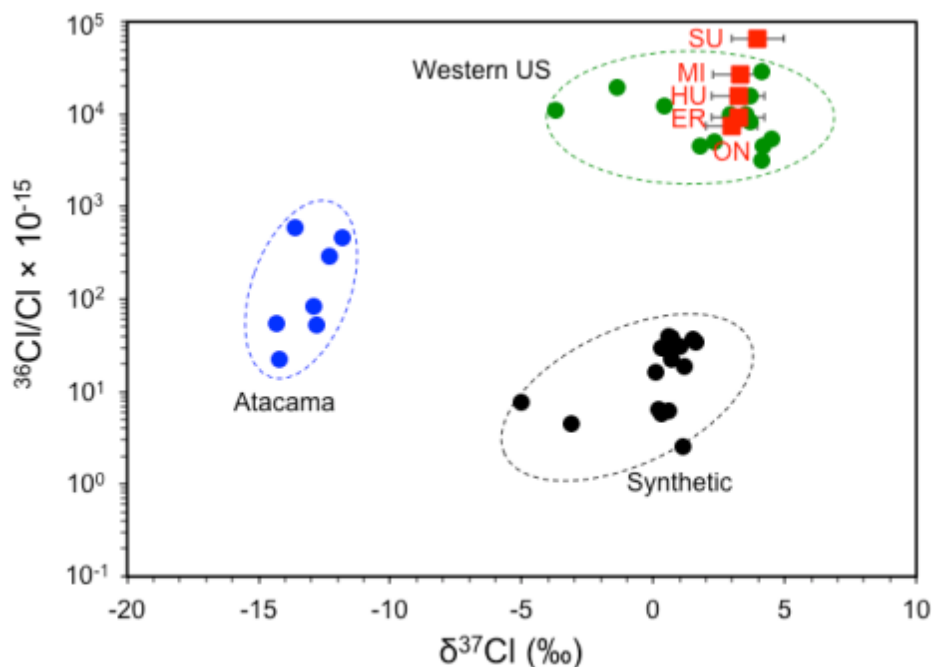


**Figure 2.2.3. Diagram comparing  $^{36}\text{Cl}/\text{Cl}$  vs.  $\delta^{37}\text{Cl}$  values for perchlorate from the Great Lakes, in comparison with synthetic perchlorate, and natural perchlorate from Atacama (Chile) and the western USA.**  $^{36}\text{Cl}/\text{Cl}$  errors are smaller than data 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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  concentrations in the lakes, whereas  $\text{ClO}_4^-$  influx from the atmospheric deposition was estimated by using a value of twice the measured average  $\text{ClO}_4^-$  concentration of  $14.1 \pm 13.5$  ng/L in rainwater (Rajagopalan et al., 2009).

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

**Table 2.2.3. Physical parameters of the Great Lakes.** Data from Chapra et al. (2009), Quinn (1982), GLERL (2012).

| Lakes    | Residence time (year) | Lake volume (km <sup>3</sup> ) | Surface Area (km <sup>2</sup> ) | Drainage area (km <sup>2</sup> ) | Over Lake Evaporation (km <sup>3</sup> /year) | Over Lake Precipitation (km <sup>3</sup> /year) | Over Land Precipitation (km <sup>3</sup> /year) |
|----------|-----------------------|--------------------------------|---------------------------------|----------------------------------|-----------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Superior | 179.8                 | 12,115                         | 81,925                          | 128,084                          | 50                                            | 65                                              | 104                                             |
| Michigan | 110.2                 | 4,947                          | 57,291                          | 115,804                          | 38                                            | 47                                              | 96                                              |
| Huron    | 21.3                  | 3,567                          | 59,560                          | 132,208                          | 39                                            | 51                                              | 117                                             |
| Erie     | 2.7                   | 499                            | 25,404                          | 60,602                           | 24                                            | 24                                              | 56                                              |
| Ontario  | 7.5                   | 1,651                          | 19,121                          | 65,118                           | 13                                            | 17                                              | 62                                              |

**Table 2.2.4. Perchlorate mass-balance model results.** Units are tonnes/year, except total ClO<sub>4</sub><sup>-</sup> in tonnes.

| Lakes    | Atmospheric ClO <sub>4</sub> <sup>-</sup> Input | Upstream ClO <sub>4</sub> <sup>-</sup> Inflow | Total ClO <sub>4</sub> <sup>-</sup> Input | ClO <sub>4</sub> <sup>-</sup> Outflow | ClO <sub>4</sub> <sup>-</sup> [Input–Outflow] | Total ClO <sub>4</sub> <sup>-</sup> (tonne) |
|----------|-------------------------------------------------|-----------------------------------------------|-------------------------------------------|---------------------------------------|-----------------------------------------------|---------------------------------------------|
| Superior | 4.8 ± 4.6                                       | n.a.                                          | 4.8 ± 4.6                                 | 4.2                                   | 0.6 ± 4.6                                     | 727                                         |
| Michigan | 4.0 ± 3.9                                       | n.a.                                          | 4.0 ± 3.9                                 | 4.5                                   | -0.5 ± 3.9                                    | 495                                         |
| Huron    | 4.7 ± 4.5                                       | 8.4                                           | 13.1 ± 4.5                                | 18.6                                  | -5.5 ± 4.5                                    | 392                                         |
| Erie     | 2.3 ± 2.2                                       | 19.2                                          | 21.5 ± 2.2                                | 15.8                                  | 5.7 ± 2.2                                     | 40                                          |
| Ontario  | 2.2 ± 2.1                                       | 15.8                                          | 18.0 ± 2.1                                | 20.7                                  | -2.7 ± 2.1                                    | 149                                         |

The apparent  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  reduction (Sturchio et al., 2007), a 20 %  $\text{ClO}_4^-$  reduction would cause about 9 ‰ increase in  $\delta^{18}\text{O}$  and +3.5 ‰ increase in  $\delta^{37}\text{Cl}$  while a 30 % reduction of  $\text{ClO}_4^-$  would increase the  $\delta^{18}\text{O}$  and  $\delta^{37}\text{Cl}$  values by about +14 ‰ and +5 ‰, respectively. However, complete biodegradation of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  removal from Lake Erie without changing the isotopic composition of residual  $\text{ClO}_4^-$ . 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  $\text{ClO}_4^-$  reduction. Alternatively, if isotopic fractionation accompanies perchlorate removal, the  $\text{ClO}_4^-$  removed and that replaced by runoff input could fortuitously have similar stable isotopic compositions.

The mass-balance model also indicates possible minor  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  contribution from other sources not accounted by the atmospheric deposition of perchlorate when assuming a value of twice the  $\text{ClO}_4^-$  concentration of  $14.1 \pm 13.5$  ng/L in rainwater. This strongly indicates that there might be a similar magnitude of  $\text{ClO}_4^-$  contribution from such unaccounted sources in Lake Erie since it is close to Lakes Huron and Ontario. However, the apparent  $\text{ClO}_4^-$  removal from Lake Erie is apparently much larger than the  $\text{ClO}_4^-$  influx from unaccounted sources.

#### 2.2.3.4 $^{36}\text{Cl}$ Abundance of Perchlorate Formed During Atmospheric Bomb Tests.

Natural  $\text{ClO}_4^-$  of recent origin has relatively high  $^{36}\text{Cl}/\text{Cl}$  ratios indicating an atmospheric source. Ratios of  $^{36}\text{Cl}/\text{Cl}$  about  $8,000 \times 10^{-15}$  were measured in  $\text{ClO}_4^-$  extracted from southwest US groundwater having  $^{14}\text{C}$  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  $^{36}\text{Cl}$  were present could be expected to have much higher  $^{36}\text{Cl}/\text{Cl}$  ratios compared with  $\text{ClO}_4^-$  that formed either before or after the bomb tests (Sturchio et al., 2009).

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

The  $^{36}\text{ClO}_4^-$  abundances in the Great Lakes may have progressively increased from that of pre-bomb conditions by addition of  $\text{ClO}_4^-$  having anomalously high  $^{36}\text{Cl}/\text{Cl}$  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 ( $\sim 180$  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)



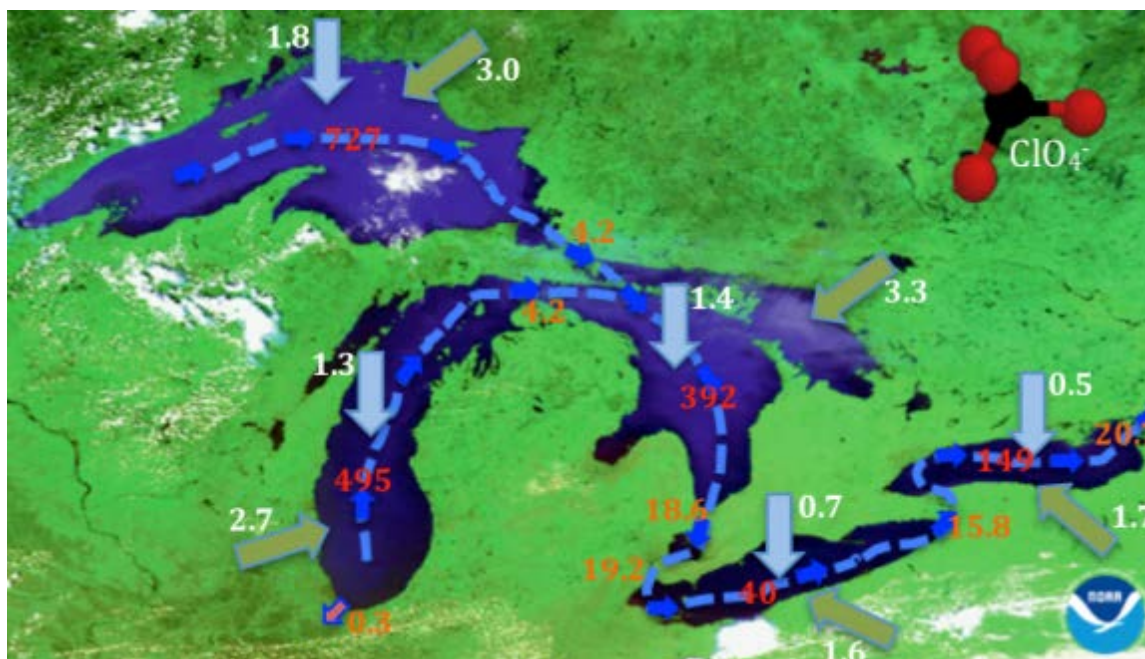


**Figure 2.2.4. Estimated perchlorate fluxes in the Great Lakes.** Red numbers represent total perchlorate inventories in each lake (tonne), white numbers next to the arrows represent perchlorate fluxes (tonne/year) from direct atmospheric deposition and from the drainage basins when assuming 14.1 ng/L  $\text{ClO}_4^-$  concentrations in the rainwater, and orange numbers represent  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  occurrences in selected globally distributed settings and an overview of the factors controlling  $\text{ClO}_4^-$  accumulation in the environment. We measured indigenous  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  and co-occurring  $\text{Cl}^-$  and  $\text{NO}_3^-$  as well as the sources and sinks of  $\text{NO}_3^-$  based on isotope data ( $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ , and  $\Delta^{17}\text{O}$ ). Our objectives were to evaluate the occurrence and fate of  $\text{ClO}_4^-$  in arid environments and the relationship of  $\text{ClO}_4^-$  to the better studied atmospherically deposited species  $\text{NO}_3^-$  and  $\text{Cl}^-$  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  $\text{ClO}_4^-$  occurrence in relation to  $\text{NO}_3^-$  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  $\text{ClO}_4^-$ , provide insights about environmental conditions controlling its distribution, and appear to indicate a new approach for evaluating arid-region biogeochemistry with particular relevance to  $\text{NO}_3^-$  processing and  $\text{Cl}^-$  cycling. Our results also may contribute to understanding the prevalence of  $\text{ClO}_4^-$  on Mars and its implications to the co-occurrence of  $\text{NO}_3^-$  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  $\text{Cl}^-$ ,  $\text{NO}_3^-$ , and  $\text{ClO}_4^-$  concentrations and a subset was evaluated for  $\text{NO}_3^-$  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  $\text{NO}_3^-$  and more recently for co-occurring  $\text{ClO}_4^-$  (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  $\text{NO}_3^-$  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 Nolokey) 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  $\text{NO}_3^-$  mineral deposits are considered to have accumulated over a period of the order of  $10^6$  to  $10^7$  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 Xiacaohu) 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  $\text{NO}_3^-$  deposits (Qin et al., 2012). The  $\text{NO}_3^-$  from this region has a wide range of stable isotopic composition, ranging from predominantly un-cycled atmospheric to predominantly biogenic  $\text{NO}_3^-$ . 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  $\text{NO}_3^-$  primarily of atmospheric origin (Michalski et al., 2005) as well as elevated concentrations of  $\text{ClO}_4^-$  (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.

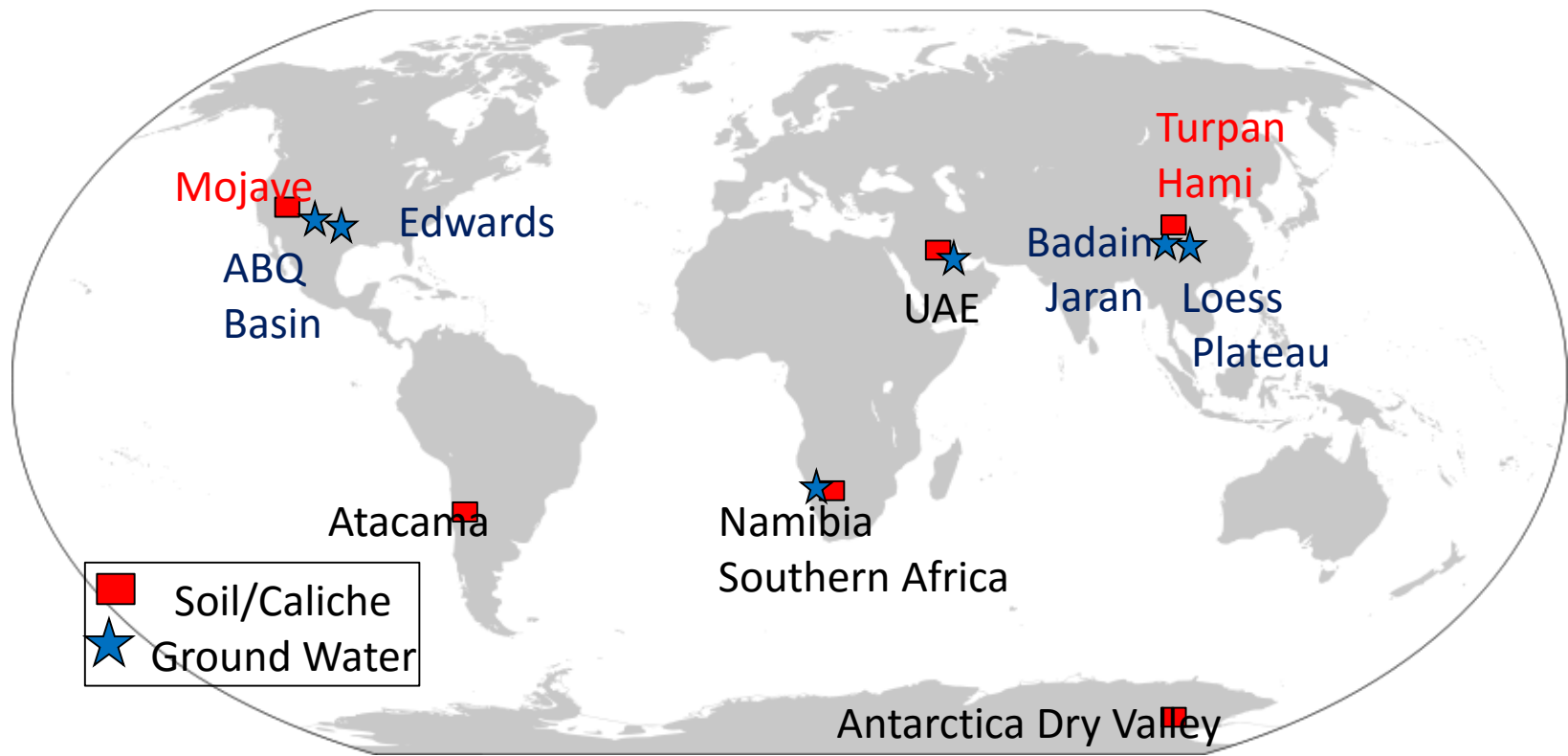


Figure 2.3.1. Map of Sample Locations

**Table 2.3.1. Locations, sample types, summary statistics for  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ , and  $\text{Cl}^-$  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)

| Location                   | Site                 | Sample Type                  | Molar Ratio                |                          |                          | $\text{NO}_3$ and $\text{ClO}_4$ |        | $\text{Cl}$ and $\text{ClO}_4$ |       | $\text{Cl}$ and $\text{NO}_3$ |       |
|----------------------------|----------------------|------------------------------|----------------------------|--------------------------|--------------------------|----------------------------------|--------|--------------------------------|-------|-------------------------------|-------|
|                            |                      |                              | $\text{NO}_3/\text{ClO}_4$ | $\text{Cl}/\text{ClO}_4$ | $\text{Cl}/\text{N O}_3$ | r                                | P      | r                              | P     | r                             | P     |
| All GW                     |                      |                              | 24,000                     | 240,000                  | 15                       | 0.98                             | <0.001 | 0.87                           | <.001 | 0.92                          | <.001 |
| United States              | Edwards Aquifer      | Public Supply/<br>Monitoring | 22,000                     | 96,000                   | 4.3                      | 0.22                             | 0.04   | 0.27                           | 0.01  | -.07                          | 0.46  |
|                            | Albuquerque Basin    | Supply/<br>Monitoring        | 22,000                     | 300,000                  | 14                       | 0.82                             | <.001  | 0.22                           | 0.13  | 0.05                          | 0.69  |
| China                      | Loess Plateau        | Springs                      | 11,000                     | 47,000                   | 5.4                      | 0.95                             | <0.001 | 0.15                           | 0.69  | 0.35                          | 0.33  |
|                            | Badain Jaran         | Shallow Wells/Springs        | 61,000                     | 1,000,000                | 17                       | 0.57                             | 0.01   | 0.37                           | 0.11  | 0.20                          | 0.4   |
| Namibia                    |                      | Springs/well                 | 29,000                     | 2,300,000                | 81                       | 0.91                             | <0.001 | 0.89                           | 0.001 | 0.96                          | <.001 |
| UAE                        |                      | Supply and Monitoring Wells  | 22,000                     | 550,000                  | 25                       | 0.98                             | <0.001 | 0.95                           | <.001 | 0.94                          | <.001 |
| Deposition                 |                      |                              |                            |                          |                          |                                  |        |                                |       |                               |       |
| United States <sup>1</sup> | 18 sites across U.S. | Wet Deposition               | 100,000                    | 35,000                   | 0.34                     | 0.7                              | <0.001 | 0.52                           | <0.01 | .5 <sup>3</sup>               | .01   |
| United States <sup>2</sup> | Mojave               | Total Deposition             | 22,000                     | 6,600                    | 0.33                     | 0.89                             | <0.01  | 0.84                           | <.01  | 0.89                          | <.01  |

**Table 2.3.2. Locations, sample types, summary statistics for  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ , and  $\text{Cl}^-$  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).

| Location                                                   | Site                  | Sample Type | Sample Depth Range (m) | Molar Ratio                                    |                                  |                                 | NO <sub>3</sub> and ClO <sub>4</sub> <sup>-</sup> |        | Cl and ClO <sub>4</sub> <sup>-</sup> |       | Cl and NO <sub>3</sub> <sup>-</sup> |        |
|------------------------------------------------------------|-----------------------|-------------|------------------------|------------------------------------------------|----------------------------------|---------------------------------|---------------------------------------------------|--------|--------------------------------------|-------|-------------------------------------|--------|
|                                                            |                       |             |                        | NO <sub>3</sub> /ClO <sub>4</sub> <sup>-</sup> | Cl/ClO <sub>4</sub> <sup>-</sup> | Cl/NO <sub>3</sub> <sup>-</sup> | r                                                 | P      | r                                    | P     | r                                   | P      |
| United States-Mojave                                       | Total                 |             |                        | 85000                                          | 580000                           | 6.7                             | 0.66                                              | <.001  | 0.18                                 | 0.06  | 0.42                                | <.001  |
|                                                            | Saratoga Hills        | Profile     | 0-0.4                  | 114000                                         | 7400000                          | 65                              | 0.14                                              | 0.84   | -0.56                                | 0.33  | -.08                                | 0.9    |
|                                                            | Bully Hill            | Profile     | 0-0.9                  | 240000                                         | 300000                           | 1.3                             | 0.87                                              | 0.06   | .79                                  | 0.06  | .98                                 | .002   |
|                                                            | Confidence Hills      | Profile     | 0-1.0                  | 115000                                         | 7400000                          | 65                              | 0.89                                              | 0.02   | 0.23                                 | 0.66  | 0.47                                | 0.35   |
|                                                            | Confidence Hills      | Profile     | 0-0.3                  | 41000                                          | 530000                           | 13                              | 0.98                                              | .002   | 0.08                                 | 0.89  | 0.04                                | 0.95   |
|                                                            | Confidence Hills      | Profile     | 0-0.7                  | 25000                                          | 6300000                          | 174                             | 0.99                                              | <0.001 | 0.72                                 | 0.17  | 0.97                                | 0.03   |
|                                                            | Confidence Hills      | Profile     | 0-0.3                  | 110000                                         | 141000                           | 1.3                             | 0.28                                              | 0.72   | 0.24                                 | 0.76  | 0.77                                | 0.23   |
|                                                            | Confidence Hills      | Profile     | 0-0.4                  | 91000                                          | 480000                           | 5.2                             | 0.30                                              | 0.62   | -0.31                                | 0.61  | 0.80                                | 0.1    |
|                                                            | Desert Pavement       | Composite   | 0-0.3                  | 86000                                          | 100000                           | 1.2                             | 0.90                                              | 0.01   | 0.81                                 | 0.05  | 0.96                                | 0.002  |
|                                                            | Desert Pavement       | Profile     | 0-0.6                  | 72000                                          | 77000                            | 1.3                             | 0.99                                              | <.001  | 0.99                                 | <.001 | 0.99                                | <0.001 |
|                                                            | Subsurface Salt Bulge | Profile     | 0-16                   | 40000                                          | 190000                           | 4.6                             | 0.92                                              | <.001  | 0.64                                 | 0.001 | 0.75                                | <.001  |
|                                                            | Rainbow Hills         | Grab        | 0-4                    | 230000                                         | 216000                           | 1.0                             | 0.99                                              | 0.002  | 0.99                                 | .001  | 0.99                                | 0.01   |
|                                                            | Fort Irwin            | Profile     |                        | 216000                                         | 709000                           | 3.53                            | 0.61                                              | 0.2    | 0.04                                 | 0.93  | 0.59                                | 0.22   |
| Southern Africa                                            | Total                 |             |                        | 120000                                         | 2700000                          | 22                              | 0.79                                              | <.001  | -.01                                 | 0.96  | <0.08                               | 0.67   |
|                                                            | Namibia               | Composite   | 0-0.3                  | 96000                                          | 730000                           | 7.3                             | 0.81                                              | <.001  | 0.07                                 | 0.77  | -0.05                               | 0.82   |
|                                                            | South Africa          | Composite   | 0-0.3                  | 150000                                         | 650000                           | 4.4                             | 0.99                                              | 0.06   | 0.93                                 | 0.25  | 0.96                                | .18    |
|                                                            | Botswana              | Composite   | 0-0.3                  | 125000                                         | 41000000                         | 330                             | 0.96                                              | <.001  | 0.52                                 | .23   | 0.53                                | .23    |
| UAE                                                        |                       |             |                        | 21000                                          | 870000                           | 42                              | 0.99                                              | <.001  | 0.54                                 | .11   | 0.25                                | 0.40   |
|                                                            | Soils                 | Composite   | 0-0.3                  | 14000                                          | 19000                            | 1.1                             | 0.87                                              | 0.05   | 0.03                                 | .96   | 0.41                                | 0.36   |
|                                                            | Sabkha                | Composite   | 0-1                    | 28000                                          | 1700000                          | 82                              | 0.99                                              | <.001  | 0.15                                 | .81   | -0.12                               | 0.8    |
| Atacama                                                    | Total                 |             |                        | 1400                                           | 1500                             | 1.1                             | 0.63                                              | <.001  | 0.26                                 | .006  | 0.66                                | <.001  |
|                                                            | AT6                   | Profile     | 0-1.5                  | 2200                                           | 4700                             | 2.3                             | 0.74                                              | 0.002  | 0.59                                 | .02   | 0.48                                | 0.08   |
|                                                            | AT8                   | Profile     | 0-2.0                  | 570                                            | 1500                             | 2.7                             | 0.65                                              | 0.002  | 0.53                                 | .02   | 0.52                                | 0.02   |
|                                                            | AT9                   | Profile     | 0-1.9                  | 730                                            | 1100                             | 1.5                             | 0.91                                              | <.001  | 0.61                                 | .08   | 0.38                                | 0.31   |
|                                                            | AT16                  | Profile     | 0-0.7                  | 13000                                          | 1300                             | 0.1                             | 0.98                                              | <.001  | 0.48                                 | .33   | -0.50                               | 0.31   |
|                                                            | AT18                  | Profile     | 0-3.0                  | 1600                                           | 1200                             | 0.7                             | 0.77                                              | <.001  | 0.74                                 | .002  | 0.93                                | <0.001 |
|                                                            | Mine                  | Profile     | 0-3.0                  | 550                                            | 615                              | 1.1                             | 0.32                                              | 0.22   | 0.86                                 | <.001 | 0.93                                | <0.001 |
|                                                            | Transect              | Composite   | 0-.3                   | 1000                                           | 2200                             | 2.1                             | 0.93                                              | <.001  | -0.1                                 | .69   | 0.2                                 | 0.44   |
| China                                                      | Turpan-Hami           | Grab        | <2                     | 12000                                          | 190000                           | 17                              | 0.61                                              | 0.05   | -.29                                 | 0.39  | -0.56                               | 0.07   |
| Antarctica<br>McMurdo<br>Dry Valleys-<br>University Valley | Total                 |             | <0.7                   | 14,000                                         | 7200                             | 0.35                            | 0.78                                              | <.001  | 0.81                                 | <.001 | 0.75                                | <.001  |
|                                                            | 1                     | Profile     | 0-.36                  | 11,000                                         | 4,600                            | 0.46                            | 0.83                                              | <.001  | 0.72                                 | .002  | 0.66                                | 0.006  |
|                                                            | 2                     | Profile     | 0-.40                  | 20,000                                         | 6,700                            | 0.34                            | 0.99                                              | <.001  | 0.99                                 | <.001 | 0.99                                | <0001  |
|                                                            | 3                     | Profile     | 0-.56                  | 7,900                                          | 5,000                            | 0.66                            | 0.98                                              | <.001  | 0.99                                 | <.001 | 0.97                                | <.001  |
|                                                            | 4                     | Profile     | 0-.66                  | 11,000                                         | 8,100                            | 0.87                            | 0.86                                              | <.001  | 0.98                                 | <.001 | 0.79                                | 0.004  |
|                                                            | 5                     | Profile     | 0-.66                  | 19,000                                         | 10,000                           | 0.68                            | 0.75                                              | 0.03   | 0.97                                 | <.001 | 0.77                                | 0.03   |
|                                                            | 7                     | Profile     | 0-.16                  | 13,000                                         | 8,100                            | 0.64                            | 0.98                                              | 0.004  | 0.82                                 | .09   | 0.76                                | 0.13   |



### 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  $\mu\text{m}$ ). All extraction sets were accompanied by an extraction duplicate and extraction sample spike (soil + known amount of  $\text{ClO}_4^-$  spike), extraction blank (DDI water only), and extraction spike (known amount of  $\text{ClO}_4^-$  spike). Moisture contents of samples were determined by drying at 105  $^\circ\text{C}$  for 24 hours. Sub-samples of some extracts were preserved at pH~11 for analysis of  $\text{NO}_3^-$  stable isotopic composition.

#### 2.3.3.2 Analytical Methods

Analysis of soil extracts and groundwater for  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ , and  $\text{NO}_3^-$  stable isotopic composition has been described previously (Section 2.2; Jackson et al., 2010). Briefly,  $\text{ClO}_4^-$  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<sup>TM</sup> triple quadrupole mass spectrometer equipped with a Turbo-IonSpray<sup>TM</sup> source. A 45 mM hydroxide ( $\text{NaOH}$ ) eluent at 0.3  $\text{ml min}^{-1}$  was followed by 90% acetonitrile (0.3  $\text{ml min}^{-1}$ ) as a post-column solvent. To overcome matrix effects, all samples were spiked with an oxygen-isotope ( $^{18}\text{O}$ ) labeled  $\text{ClO}_4^-$  internal standard. A 25  $\mu\text{L}$  loop was used for sample loading with a 0.0005  $\mu\text{M}$  level of quantification. Chloride and  $\text{NO}_3^-$  were analyzed following EPA Method 300.0 using a Dionex LC20, an IonPac AS14A column (4 X 250 mm), 8 mM  $\text{Na}_2\text{CO}_3$ /1 mM  $\text{NaHCO}_3$  eluent, and an Anion Atlas electrolytic suppressor. The limit of detection (LOD) of  $\text{Cl}^-$  and  $\text{NO}_3^-$  were 1.4  $\mu\text{M}$  and 3.5  $\mu\text{M}$ , respectively. Individual sample quantification limits were based on the final dilution of the sample extract.

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

For selected samples, the  $\Delta^{17}\text{O}$  value of  $\text{NO}_3^-$  was measured by dual-inlet isotope-ratio analysis of  $\text{O}_2$  produced by off-line partial decomposition of  $\text{AgNO}_3$  (modified from Michalski et al.,

2002).  $\text{NO}_3^-$  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<sub>3</sub> 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<sub>3</sub> salt. The AgNO<sub>3</sub> was heated under vacuum at 520°C while connected to a 5A° mol-sieve trap cooled with liquid N<sub>2</sub> to collect O<sub>2</sub>, which was then isolated and transferred to the mass spectrometer and analyzed against tank O<sub>2</sub>. Measured  $\Delta^{17}\text{O}$  values of  $\text{NO}_3^-$  isotopic reference materials RSIL-N11 and USGS35 prepared as AgNO<sub>3</sub> 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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ . 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 $\text{ClO}_4^-$ , $\text{NO}_3^-$ Concentrations and $\text{NO}_3^-$ Isotopes

Combined data from this study indicate the global occurrence of  $\text{ClO}_4^-$  and positive correlation between  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  to atmospheric production and subsequent deposition (Section 2.1 and 2.2; Rajagopalan et al., 2009; Jackson et al., 2010). Excluding the Atacama site, our results are generally consistent with this assertion based on similarities between site  $\text{NO}_3^-/\text{ClO}_4^-$  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 (Rajagopalan 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  $\text{NO}_3^-/\text{ClO}_4^-$  in wet deposition ranged over ~one order of magnitude, while  $\text{Cl}^-/\text{ClO}_4^-$  ratios varied over 2 orders of magnitude and were significantly related to distance from a coast.  $\text{NO}_3^-/\text{ClO}_4^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  molar ratios in total deposition were lower than in wet deposition and the  $\text{NO}_3^-$  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  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  at most sites, concentrations of  $\text{Cl}^-$  appear to be relatively poorly correlated with either  $\text{NO}_3^-$  or  $\text{ClO}_4^-$  for most sites (Figures 2.3.4 and 2.3.5). Most samples had relatively high  $\text{Cl}^-/\text{NO}_3^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  ratios

compared to those of modern precipitation and total deposition in North America (Figure 2.3.7). Local high deposition fluxes of marine (seasalt)  $\text{Cl}^-$  could account for some, but not all of these differences. Additional sources of  $\text{Cl}^-$  unrelated to the  $\text{Cl}^-$  deposited with  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  could include local  $\text{Cl}^-$  atmospheric inputs remobilized from salt flats, upwelling of saline groundwater, and run-on of  $\text{Cl}^-$  laden water. Alternatively, high  $\text{Cl}^-/\text{NO}_3^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  ratios could indicate simultaneous net loss of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  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.  $\text{Cl}^-$  remaining after such episodic reduction is hereafter referred to as “episodic  $\text{Cl}^-$ ”. Alternatively, oxyanions could have been depleted relative to  $\text{Cl}^-$  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  $\text{Cl}^-$  not associated with the current  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  as “non-co-deposited  $\text{Cl}^-$ ”.

Isotope data indicate sources of  $\text{NO}_3^-$  from these sites varied from essentially 100% unaltered atmospheric to 100% biogenic, where biogenic  $\text{NO}_3^-$  could be derived from various N sources including recycled atmospheric  $\text{NO}_3^-$  (Figure 2.3.6). Collectively, isotopic analyses of  $\text{NO}_3^-$  are consistent with two major factors affecting  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$ : (1) variation in the relative proportions of atmospheric  $\text{NO}_3^-$  (low  $\delta^{15}\text{N}$ , high  $\delta^{18}\text{O}$ ) and biogenic  $\text{NO}_3^-$  (high  $\delta^{15}\text{N}$ , low  $\delta^{18}\text{O}$ ), yielding an overall negative correlation between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , and (2) variation in the isotope fractionation effect of  $\text{NO}_3^-$  reduction, yielding local positive correlations between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  at some sites.  $\text{NO}_3^-$  reduction can occur in the unsaturated zone and/or in groundwater after recharge. In addition, minor variations in  $\delta^{18}\text{O}$  of biogenic  $\text{NO}_3^-$  may be related to local  $\delta^{18}\text{O}$  of meteoric water during nitrification, and N cycling in soils and plants can cause local or regional variation in the  $\delta^{15}\text{N}$  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  $\text{NO}_3^-$  reduction effects appear to be most prevalent at sites with relatively large fractions of biogenic  $\text{NO}_3^-$ , 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  $\delta^{15}\text{N}$ .

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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  with the isotopic indicators of  $\text{NO}_3^-$  sources, based on the assumption that atmospheric sources ultimately are important for both  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ . Assuming no net gains or losses of  $\text{ClO}_4^-$  other than atmospheric deposition, relations between  $\text{NO}_3^-/\text{ClO}_4^-$  ratios and  $\text{NO}_3^-$   $\delta^{18}\text{O}$  values can be explored using a mixing model with three bounding cases (Figure 2.3.8): 1) deposition and preservation of atmospheric  $\text{NO}_3^-$  with no addition of biogenic  $\text{NO}_3^-$ ; 2) preservation of atmospheric  $\text{NO}_3^-$  with addition of biogenic  $\text{NO}_3^-$  produced by nitrification of reduced N from either fixed  $\text{N}_2$ , atmospheric  $\text{NH}_4^+$ , or accumulated organic matter (Figure 2.3.8 black dashed lines); and 3) assimilation of atmospheric  $\text{NO}_3^-$  and regeneration with biogenic  $\text{NO}_3^-$  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  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  reduction or  $\text{NO}_3^-$  reduction alone, which are alternative possibilities discussed below. For the Mojave and Atacama sites,  $\text{ClO}_4^-$  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  $\text{H}_2\text{O}$  as an alternative to nitrification as a way of lowering  $\delta^{18}\text{O}$  of atmospheric  $\text{NO}_3^-$ , but there is no direct evidence for such exchange in these environments, whereas there is evidence that lower  $\text{NO}_3^-$   $\delta^{18}\text{O}$  values are associated with increasing soil biologic activity.

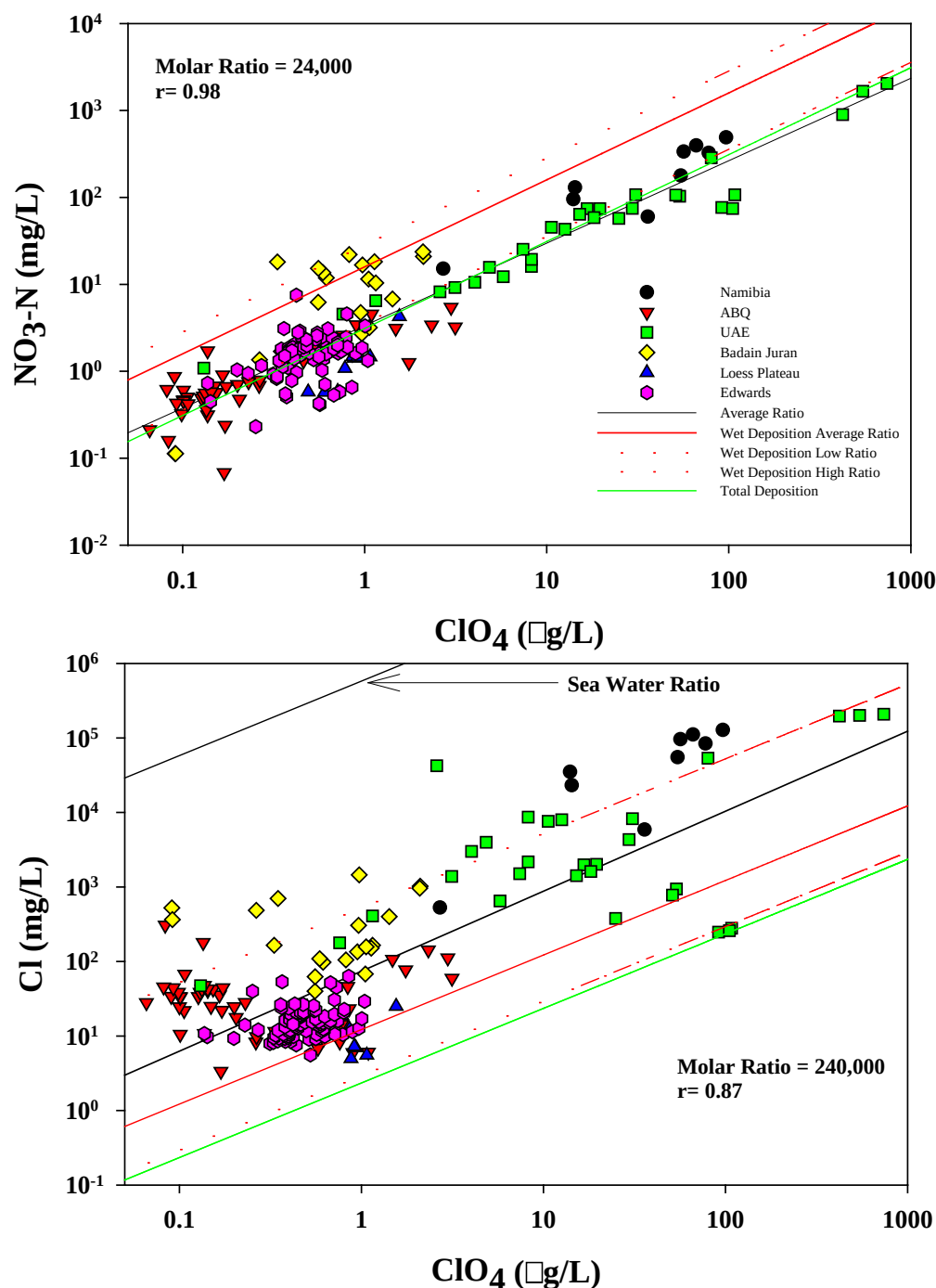


**Figure 2.3.2. Relations among  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ , and  $\text{Cl}^-$  concentrations in groundwater.** Ratios for all groundwater samples are shown by solid black lines. Average ratios in U.S. wet deposition are shown as solid red lines, and minimum and maximum average site ratios 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 (Rajagopalan et al., 2009). The total deposition ratio (green line) represents the 6 year average of quarterly samples (Andraski et al., 2014).

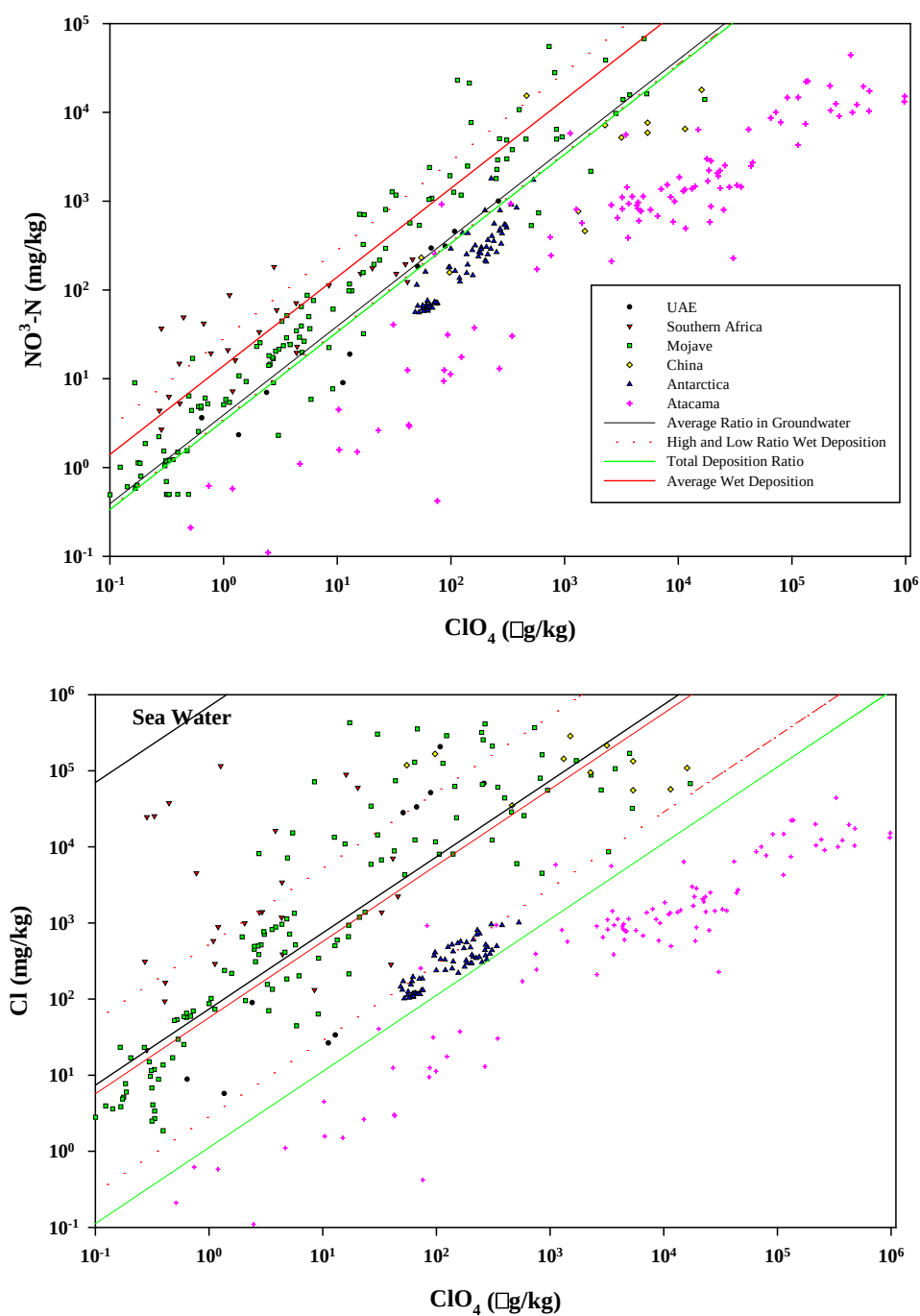


**Figure 2.3.3. Relations between concentrations of  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  and  $\text{Cl}^-$  for all soils and caliches.** Average ratio in wet deposition is shown as solid red lines, and average minimum and maximum ratios for all sites are shown as dashed lines. Ratios for wet deposition include 18 sites located across the conterminous U.S., Alaska, and Puerto Rico for weekly samples over a three year period (Rajagopalan et al., 2009). The total deposition ratio (green line) represents the 6 year average of quarterly samples (Andraski et al., 2014).

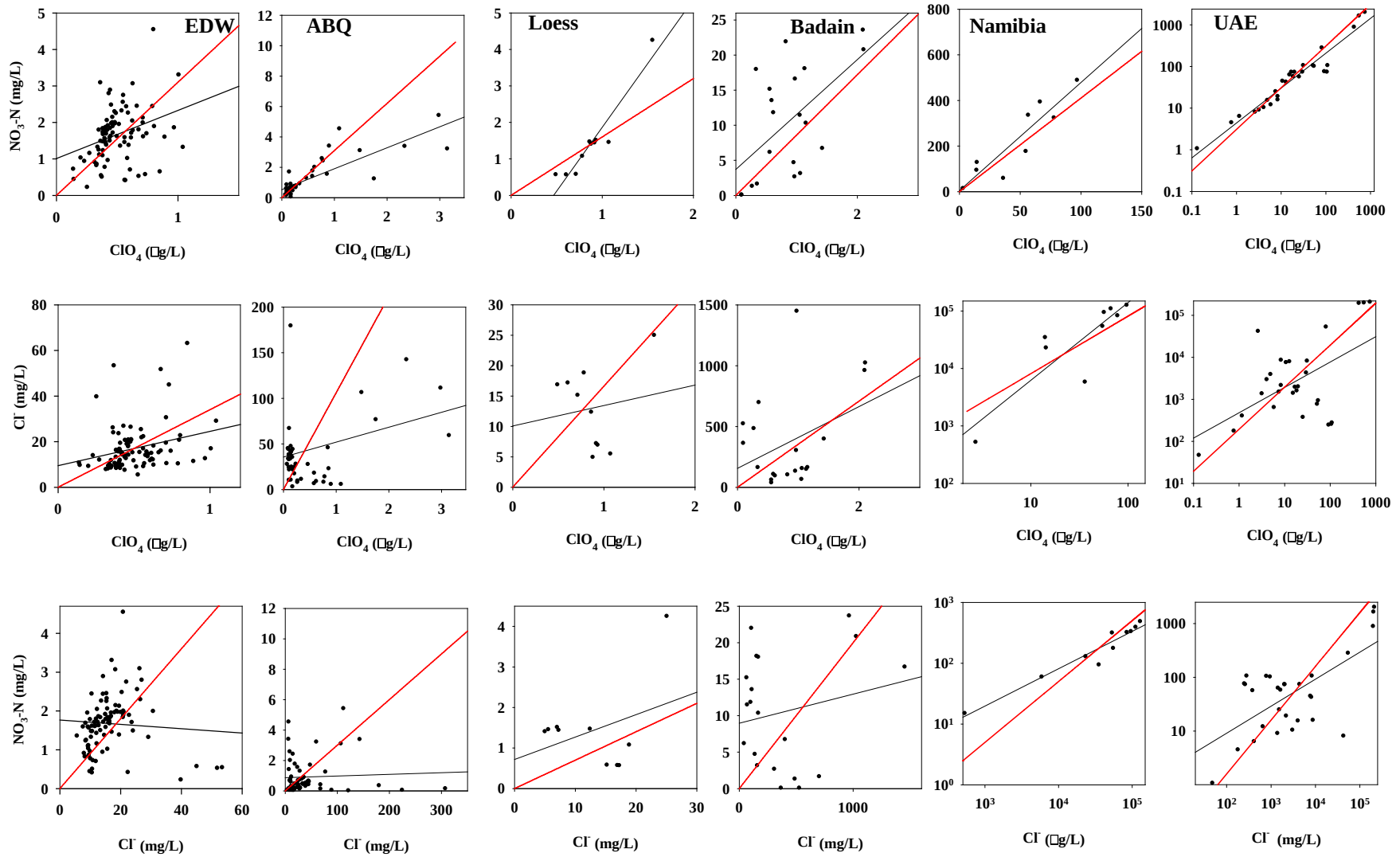


**Figure 2.3.4. Relations among  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ , and  $\text{Cl}^-$  in groundwater from arid and semi-arid locations.** Solid black lines represent linear regressions of concentration data; red lines represent averages of log-transformed ratios. Correlation coefficients and P values are listed in Table 2.3.1.

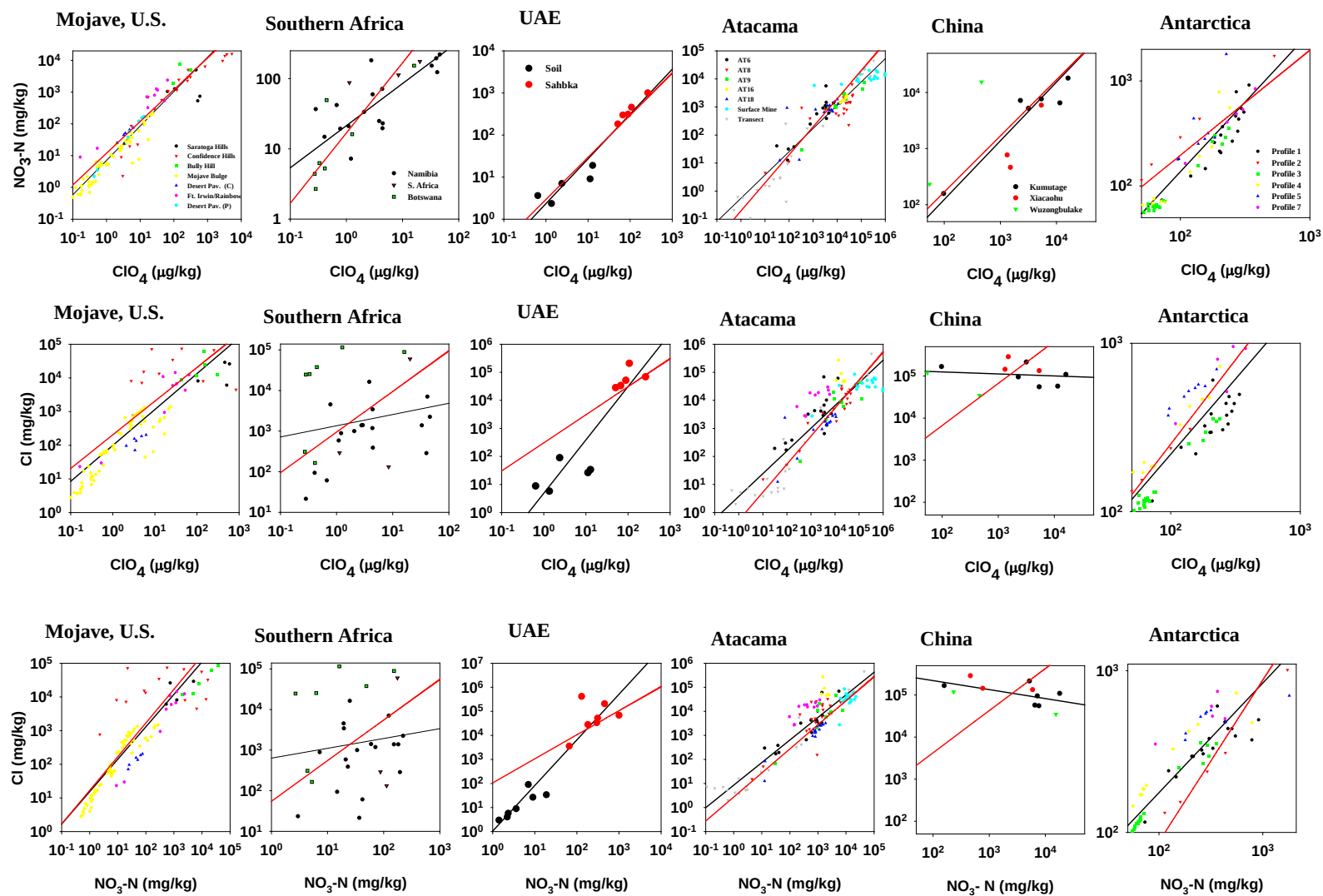


**Figure 2.3.5. Relations among  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ , and  $\text{Cl}^-$  concentrations in soils and caliches from arid and semi-arid locations.** Solid black lines represent linear regressions of concentration data; red lines represent averages of log –transformed ratios. Correlation coefficients and P values are listed in Table 2.3.2.



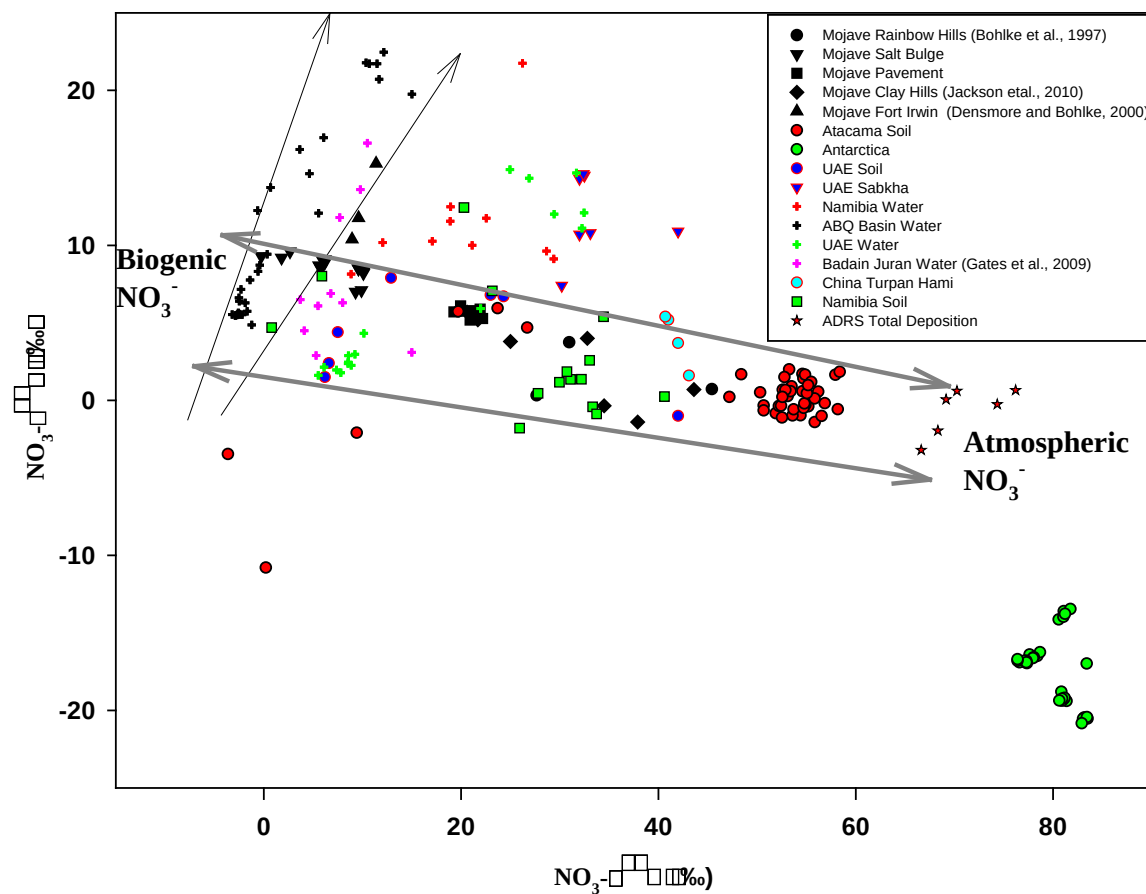


**Figure 2.3.6. Relation between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  in groundwater and leachate from soil and caliche samples.** Black arrows represent the range of slopes reported previously for isotopic fractionation due to denitrification and grey arrows represent general trends in mixing ratios of atmospheric and biogenic  $\text{NO}_3^-$ .

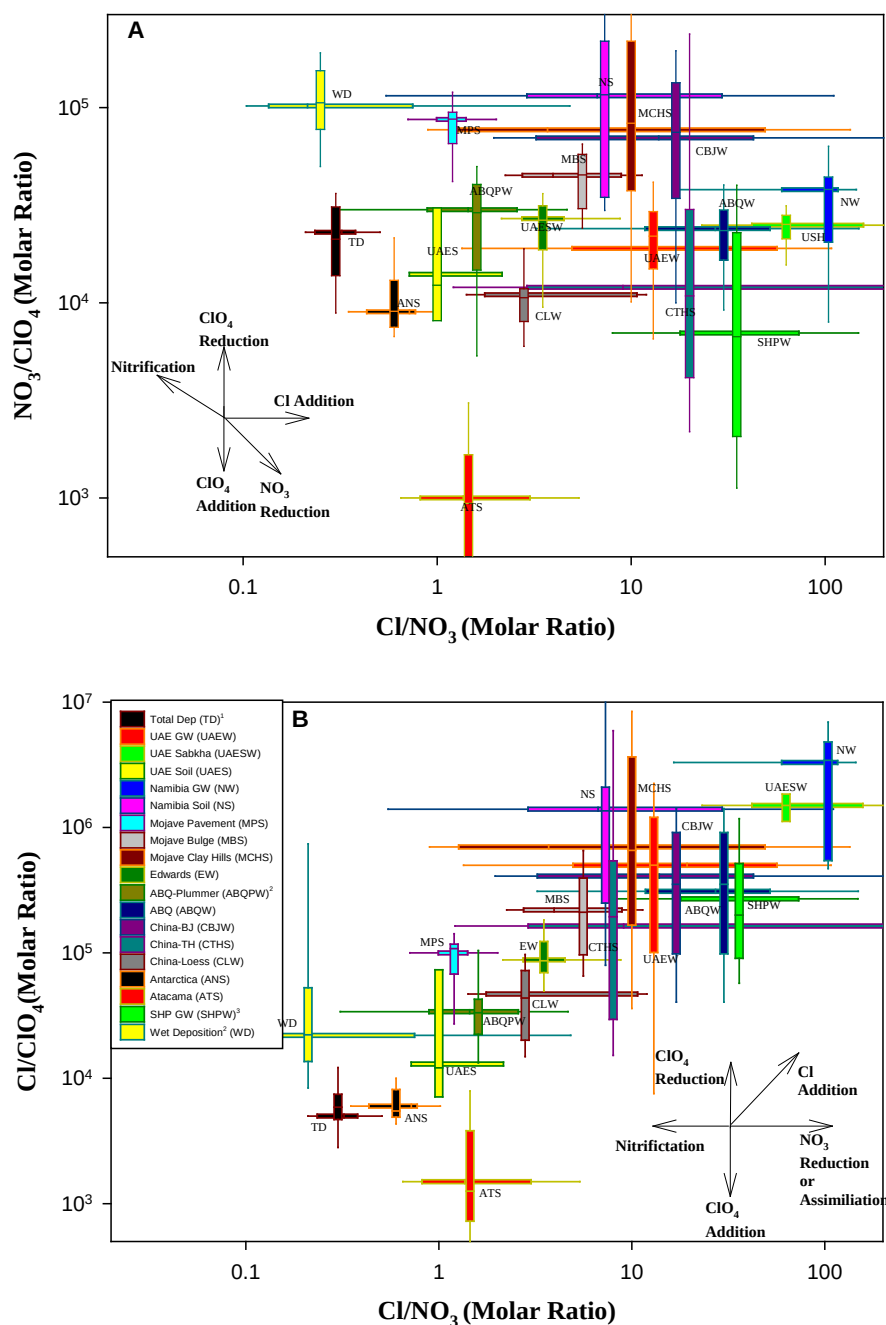


**Figure 2.3.7. Variation of (A) NO<sub>3</sub>/ClO<sub>4</sub> or (B) Cl/CIO<sub>4</sub> ratios with respect to Cl/NO<sub>3</sub> ratios in soils and groundwaters from arid and semi-arid locations as well as wet and dry deposition in North America. Box plots represent the 25<sup>th</sup> and 75<sup>th</sup> percentile values and extended lines from boxes represent the 90<sup>th</sup> and 10<sup>th</sup> percentiles. Arrows indicate hypothetical qualitative trajectories that could be caused by various processes. (<sup>1</sup>Andraski et al., 2104; <sup>2</sup>Plummer et al., 2006; <sup>3</sup>Rajagopalan et al., 2006).**

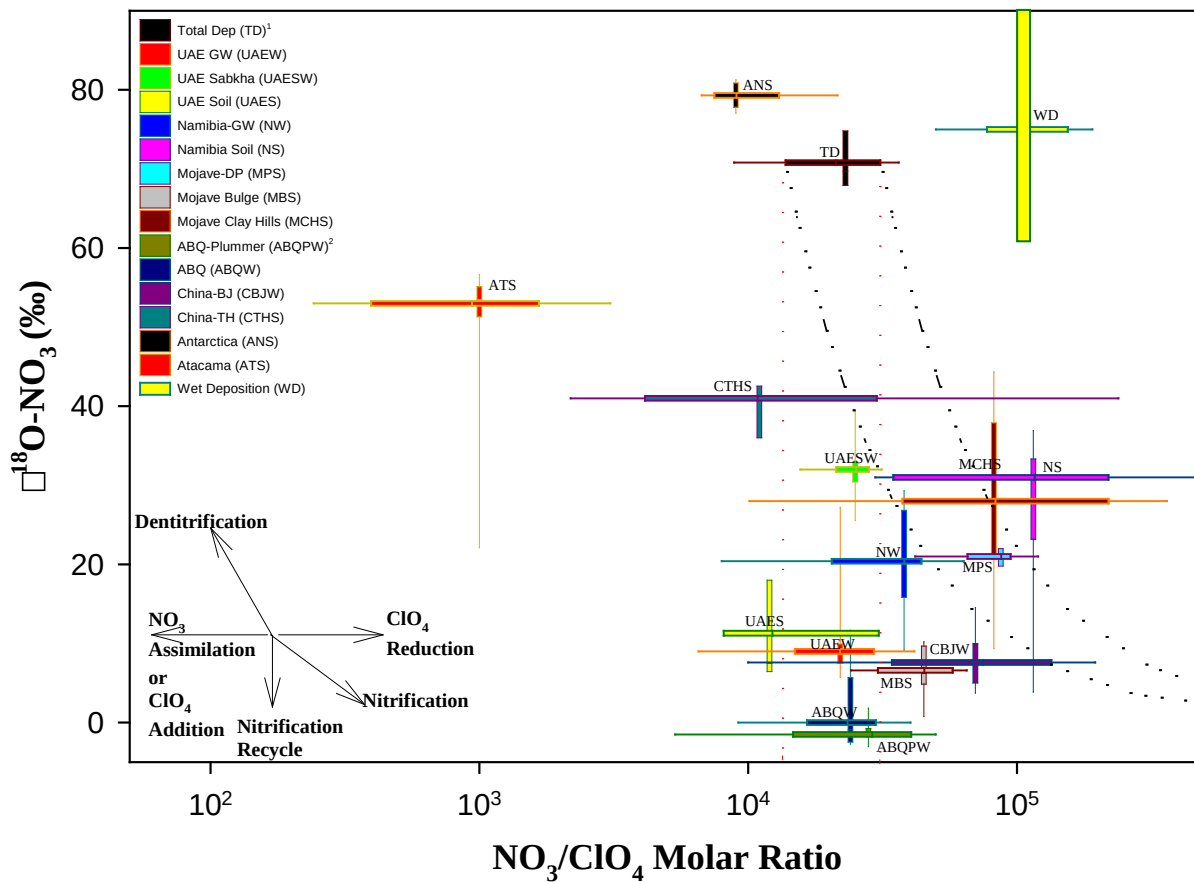


**Figure 2.3.8. Relation between  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  and bulk  $\text{NO}_3/\text{ClO}_4$  ratios in soils and groundwaters from arid and semi-arid locations as well as wet and dry deposition in North America.** Box plots represent the 25th and 75th percentile values and extended lines from boxes represent the 90<sup>th</sup> and 10<sup>th</sup> percentiles. Dashed curves originating at total deposition indicate hypothetical trends caused by either: 1 (red lines) atmospheric  $\text{NO}_3$  assimilation, mineralization, and nitrification with no net loss or gain of N; or 2 (black curve) addition of biogenic  $\text{NO}_3$  to uncycled atmospheric  $\text{NO}_3$ , assuming in both cases  $\text{ClO}_4$  is unaffected. Arrows in the lower left corner indicate hypothetical qualitative trajectories that could be caused by various processes. (<sup>1</sup>Andraski et al., 2014; <sup>2</sup>Plummer et al., 2006).

### 2.3.5.2 Groundwater Results

#### **Groundwater Overview**

The  $\text{ClO}_4^-$  concentrations in groundwater samples varied over four orders of magnitude, whereas the  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{NO}_3^-$  reduction in groundwater was not associated with substantial variation of  $\text{NO}_3^-/\text{ClO}_4^-$  ratios. However, it should be noted that the variation in  $\text{NO}_3^-/\text{ClO}_4^-$  ratios within most sites could be sufficient to accommodate substantial (>50%)  $\text{NO}_3^-$  losses.  $\text{Cl}^-/\text{ClO}_4^-$  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  $\text{Cl}^-/\text{ClO}_4^-$  ratios compared to total deposition for non-coastal sites supports addition of  $\text{Cl}^-$  from non-atmospheric sources, “episodic  $\text{Cl}^-$ ” or “non-co-deposited  $\text{Cl}^-$ ” in many cases.

#### **Albuquerque Basin, U.S. (ABQ)**

$\text{ClO}_4^-$  concentrations in ABQ groundwater samples ( $n=63$ ) varied over a relatively narrow range ( $< 0.05$  ( $n=22$ ) to  $3.1 \mu\text{g/L}$ ) 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  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  were correlated ( $r = 0.82$ ) but there was no correlation between  $\text{Cl}^-$  and either  $\text{ClO}_4^-$  or  $\text{NO}_3^-$  ( $r = 0.22$  and  $0.05$ , respectively) (Table 2.3.1 and Figure 2.3.4). The average  $\text{NO}_3^-/\text{ClO}_4^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  molar ratios were 22,000 and 300,000, respectively.

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

similar  $\text{NO}_3^-$  isotopic composition ( $\delta^{18}\text{O} \sim -1 \text{ ‰}$ ) and  $\text{NO}_3^-/\text{ClO}_4^-$  ratios (18,000 and 28,000) for two oxic groundwater samples from the ABQ basin.

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

### ***Edwards Aquifer, U.S.***

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

### ***Loess Plateau, China***

$\text{ClO}_4^-$  concentrations in Loess Plateau groundwater samples ( $n=10$ ) varied over a range ( $0.6\text{-}1.6 \text{ } \mu\text{g/L}$ ) similar to those for the ABQ basin and Edwards Aquifer (Figure 2.3.4).  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  concentrations were correlated ( $r=0.95$ ) but  $\text{Cl}^-$  was not correlated with either  $\text{ClO}_4^-$  or  $\text{NO}_3^-$  (Table 2.3.1). The  $\text{NO}_3^-/\text{ClO}_4^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  molar ratios were the lowest of any groundwater evaluated ( $\sim 11,000$  and  $47,000$ , respectively). No data are available concerning the age of the groundwater or the origin of the  $\text{NO}_3^-$  but the  $\text{NO}_3^-/\text{ClO}_4^-$  ratios are less and  $\text{Cl}^-/\text{ClO}_4^-$  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  $\text{NO}_3^-$ . Irrigation was almost absent in the area. It is not possible to discuss the origin or subsequent fate of  $\text{NO}_3^-$  due to the absence of  $\text{NO}_3^-$  isotope data, but the relation between  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  is similar to that in other geographically distant sites.

### ***Badain Jaran, China***

$\text{ClO}_4^-$  concentrations in groundwater samples ( $n=20$ ) ranged from 0.3 to 2.1  $\mu\text{g/L}$  and were correlated with  $\text{NO}_3^-$  ( $r = 0.57$ ) (Figure 2.3.4, Table 2.3.1) but  $\text{Cl}^-$  was not correlated with  $\text{ClO}_4^-$  or  $\text{NO}_3^-$ . The average  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{ClO}_4^-$  is indigenous (Gates et al., 2008a,b).  $\text{NO}_3^-$  in this system previously has been attributed to nitrification of  $\text{NH}_4^+$  derived from atmospheric deposition or from mineralization of organic matter produced by nitrogen fixation or biologic assimilation of atmospheric N. In a few samples,  $\text{NO}_3^-$  may have been partially reduced through denitrification and a few samples with elevated  $\delta^{18}\text{O}$  and low  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  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  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$  stable isotope data were available for 10 (Figure 2.3.6). Six samples previously identified as most likely to contain  $\text{NO}_3^-$  impacted by denitrification have  $\text{NO}_3^-/\text{ClO}_4^-$  molar ratios that are not significantly lower than the overall average (i.e., do not appear to indicate substantial preferential  $\text{NO}_3^-$  loss). Three samples appear to be impacted by denitrification based on relatively low  $\text{NO}_3^-/\text{Cl}^-$  ratios and elevated  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values (Gates et al., 2008a). However, even for these samples the ratio of  $\text{NO}_3^-/\text{ClO}_4^-$  is not consistently lower than the overall average.

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

### ***Namibia***

$\text{ClO}_4^-$  concentrations in Namibia groundwater samples ( $n = 10$ ) ranged from 2.7-97  $\mu\text{g/L}$  (Figure 2.3.4).  $\text{ClO}_4^-$  was correlated with  $\text{NO}_3^-$  and  $\text{Cl}^-$  ( $r = 0.91$  and  $0.89$ , respectively) and as such  $\text{NO}_3^-$  and  $\text{Cl}^-$  were also correlated ( $r=0.96$ ) (Table 1). Given the remote location of the sample sites, high concentrations of  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ , and  $\text{NO}_3^-$  isotopic composition, the  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  are considered to be natural. The average  $\text{NO}_3^-/\text{ClO}_4^-$  ratio (29,000) was similar to those in all other groundwaters evaluated excluding the Badain Jaran, while the average  $\text{Cl}^-/\text{ClO}_4^-$  molar ratio was much higher (2,300,000). Stable isotope data indicate  $\text{NO}_3^-$  in the Namibia samples was predominantly biogenic, but included substantial unaltered atmospheric components based on elevated  $\Delta^{17}\text{O}$  values in some samples (1.1 to 5.9 ‰;  $n = 5$ ). The  $\text{NO}_3^-$  also appears to have been variably affected by  $\text{NO}_3^-$  reduction based on elevated  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  values (Figure 2.3.6) and higher  $\delta^{18}\text{O}$  values than expected from biogenic-atmospheric mixtures based on the  $\Delta^{17}\text{O}$  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  $\text{NO}_3^-$ ) but altered by some denitrification (Figure 2.3.8). High  $\text{Cl}^-/\text{ClO}_4^-$  ratios could be due to high local  $\text{Cl}^-$  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  $\text{Cl}^-$  and  $\text{ClO}_4^-$  for this site but not others, and by the similar  $\text{Cl}^-/\text{ClO}_4^-$  ratios in surface soils (Figure 2.3.7).

### ***UAE***

Maximum  $\text{ClO}_4^-$  concentrations for UAE groundwater were the highest of any groundwater evaluated (Figure 2.3.4). Highest concentrations (500-740  $\mu\text{g/L}$ ) occurred in sabkha areas ( $n = 5$ ) but concentrations in some inland fresh groundwater ( $n=20$ ) were also elevated (0.1 to 108  $\mu\text{g/L}$ ), similar to the concentration range for Namibia groundwater.  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  were well correlated with each other ( $r = 0.98$ ) and with  $\text{Cl}^-$  (Table 1). The average  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{NO}_3^-$  in most ( $n=10$ ) of the fresh-water samples (production wells) for which  $\text{NO}_3^-$  stable isotope data are available ( $n=12$ ) appears to be predominantly biogenic, with little evidence of  $\text{NO}_3^-$  reduction ( $\delta^{15}\text{N} \sim 2 - 6 \text{‰}$  and  $\delta^{18}\text{O} \sim 5 - 10 \text{‰}$ ; 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  $\text{NO}_3^-$  reduction based on  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  (Figure 2.3.6) as well as  $\Delta^{17}\text{O}$  of  $\text{NO}_3^-$  (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  $\text{NO}_3^-$  and only limited loss of the atmospheric  $\text{NO}_3^-$  component if the impact of  $\text{NO}_3^-$  reduction indicated by isotope data is taken into account.  $\text{Cl}^-/\text{ClO}_4^-$  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  $\text{Cl}^-$  and  $\text{ClO}_4^-$  (Table 2.3.1, Figure 2.3.7). UAE non-sabkha groundwater is more consistent with  $\text{NO}_3^-$  cycling rather than simple dilution (addition only) by biogenic  $\text{NO}_3^-$ .  $\text{Cl}^-/\text{ClO}_4^-$  ratios are similar to sabkha samples but much higher than for UAE surface soil samples (see below) suggesting that atmospheric deposition may not be the dominant source of the  $\text{Cl}^-$ .

### **2.3.5.3 Soil and Caliche Results**

#### ***Soil and Caliche Overview***

$\text{ClO}_4^-$  concentrations in soils/caliches for all sites vary over a larger range (0.1 to  $10^6 \mu\text{g/kg}$ ) than those in groundwater (Figure 2.3.2 and 2.3.3). Similarly,  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  concentrations within sites and between sites.

Overall there appear to be major groupings of  $\text{NO}_3^-/\text{ClO}_4^-$  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 ( $\sim 100,000$ ) in wet deposition (Rajagopalan 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  $\text{NO}_3^-/\text{ClO}_4^-$  ratio, are also the most arid. Soil  $\text{Cl}^-/\text{ClO}_4^-$  ratios are much more variable than  $\text{NO}_3^-/\text{ClO}_4^-$  ratios, as in the groundwater sample sets; only the Antarctica and Atacama soil data sets exhibit a clear relation between  $\text{Cl}^-$  and  $\text{ClO}_4^-$ . 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  $\text{ClO}_4^-$  in these samples ranged from a low of 0.1  $\mu\text{g}/\text{kg}$  (salt bulge) to a high of 5,500  $\mu\text{g}/\text{kg}$  (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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  concentrations (0.1 to 20  $\mu\text{g}/\text{kg}$ ), 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  $\text{ClO}_4^-$  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  $\text{km}^2$  hill-slope area of a nearby knoll was reported to have an average  $\text{ClO}_4^-$  concentration of  $3.0 \pm 2.6 \mu\text{g}/\text{kg}$  with a range of 0.8 to 11.8  $\mu\text{g}/\text{kg}$  (Andraski et al., 2014).

$\text{ClO}_4^-$  concentrations for all sites in the Mojave Desert are significantly correlated with  $\text{NO}_3^-$  ( $r = 0.66$ ) but not with  $\text{Cl}^-$  ( $r = 0.18$ ) (Table 2.3.2, Figure 2.3.5).  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  were correlated ( $r$



= 0.90, 0.99, and 0.92, respectively).  $\text{ClO}_4^-$  was not correlated with  $\text{Cl}^-$  in the Mojave sample sets except for the subsurface salt bulge, desert pavement sites, and samples from Rainbow Hills.  $\text{Cl}^-$  and  $\text{NO}_3^-$  were also correlated for many of the same locations for which  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  were correlated. A previous study found a significant but weaker relationship between  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  detection limit (165  $\mu\text{g/kg}$ ).

The molar ratios of  $\text{NO}_3^-/\text{ClO}_4^-$  for all Mojave locations varied from 25,000 to 240,000 with an overall average of 85,000 (Table 2.3.2).  $\text{NO}_3^-/\text{ClO}_4^-$  molar ratios were most variable in the clay hills samples, while the desert pavement and sub-surface salt bulge  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\sim 100$  km of the remaining Mojave sites. In contrast to the relatively similar average  $\text{NO}_3^-/\text{ClO}_4^-$  ratios in Mojave sample sets, average  $\text{Cl}^-/\text{ClO}_4^-$  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  $\text{NO}_3^-$  ( $\delta^{18}\text{O}$ ,  $\Delta^{17}\text{O}$ , and  $\delta^{15}\text{N}$ ) in samples from the Death Valley clay hills and Rainbow Hills previously demonstrated that the  $\text{NO}_3^-$  is partially (20-50%) atmospheric in origin and partially biogenic, with no evidence of substantial isotope effect from  $\text{NO}_3^-$  reduction (Böhlke et al., 1997; Michalski et al., 2004; Jackson et al., 2010; Lybrand et al., 2013).  $\text{NO}_3^-$  in soil samples from Irwin Basin was mainly biogenic (Figure 2.3.6) (Densmore and Böhlke, 2000). The  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  in samples from the desert salt bulge support a largely biogenic source of  $\text{NO}_3^-$  while those in samples from near-by desert pavement appear to have a substantial (up to  $\sim 20\%$ ) atmospheric component (Figure 2.3.6) (Andraski et al., 2014).

$\text{NO}_3^-/\text{ClO}_4^-$  ratios and  $\text{NO}_3^-$  stable isotope ratios for samples from the Mojave clay hills are consistent with a model of atmospheric deposition and addition of biogenic  $\text{NO}_3^-$  without significant recycling (Figure 2.3.8), and with substantial addition of  $\text{Cl}^-$  (Figure 2.3.7) from “episodic  $\text{Cl}^-$ ”, “non-co-deposited  $\text{Cl}^-$ ” 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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  component and addition of biogenic  $\text{NO}_3^-$ .

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

= 0.87) and sabkha sediments ( $r = 0.97$ ) with an overall correlation coefficient of 0.99 (Table 2.3.2 and Figure 2.3.5).  $\text{Cl}^-$  and  $\text{ClO}_4^-$  were not correlated, nor were  $\text{NO}_3^-$  and  $\text{Cl}^-$ .  $\text{NO}_3^-/\text{ClO}_4^-$  molar ratios were similar in soils and sabkha sediments (14,000 and 28,000, respectively), while  $\text{Cl}/\text{ClO}_4^-$  ratios were much more variable (19,000 and 1,700,000, respectively). Isotope data indicate the source of  $\text{NO}_3^-$  in the soil samples is mainly biogenic except for two samples that may have around 20-50% unaltered atmospheric  $\text{NO}_3^-$ , both of which had very low  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  concentrations (Figure 2.3.6).  $\text{NO}_3^-$  in the sabkha samples apparently had substantial fractions of unaltered atmospheric  $\text{NO}_3^-$  (approximately 20-50 %), and it also exhibited isotopic evidence of  $\text{NO}_3^-$  reduction.  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{NO}_3^-/\text{ClO}_4^-$  ratios and  $\text{NO}_3^-$  isotopic compositions of the soil samples are consistent with substantial  $\text{NO}_3^-$  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  $\text{NO}_3^-/\text{ClO}_4^-$  ratios and expected impact of  $\text{NO}_3^-$  reduction. Interestingly, UAE soil  $\text{Cl}/\text{ClO}_4^-$  ratios are near Mojave total deposition values and an order of magnitude lower than UAE groundwater values despite the similar  $\text{NO}_3^-/\text{ClO}_4^-$  ratios and proximity to the coast. The lack of correlation between soil  $\text{Cl}^-$  and  $\text{ClO}_4^-$ , but strong correlation for groundwater coupled with higher  $\text{ClO}_4^-$  concentrations in groundwater than soil, suggest that the groundwater  $\text{Cl}^-$ ,  $\text{NO}_3^-$ , and  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  (~100 to 16,000  $\mu\text{g}/\text{kg}$ ) were present in a relatively small set ( $n = 11$ ) of samples from three sites in the vicinity of recently described massive  $\text{NO}_3^-$  deposits of northern China (Qin et al., 2012; Li et al., 2010). Concentrations of  $\text{ClO}_4^-$  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  $\text{NO}_3^-$  deposits, even though the  $\text{NO}_3^-$  concentrations were similar between the Atacama and China deposits (Figure 2.3.5). Turpan-Hami  $\text{NO}_3^-$  has been attributed largely to long-term atmospheric deposition, based on elevated  $\Delta^{17}\text{O}$  (5-20‰) and  $\delta^{18}\text{O}$  (30-60‰) values (Qin et al., 2012; Li et al., 2010), which are intermediate to  $\text{NO}_3^-$  from the Mojave clay hills and Atacama Desert. Previously reported ranges of  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  indicate the Turpan-Hami  $\text{NO}_3^-$  is approximately 25-75% unaltered atmospheric. Data from the current study indicate similar atmospheric components (Figure 2.3.6).  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  were correlated ( $r = 0.61$ ) but  $\text{Cl}^-$  and  $\text{ClO}_4^-$ , and  $\text{Cl}^-$  and  $\text{NO}_3^-$ , were not.  $\text{Cl}^-$  concentration varied relatively little, while  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  concentrations varied over 2 orders of magnitude (Figure 2.3.5). Molar ratios of  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{NO}_3^-$  component and the relatively low  $\text{NO}_3^-/\text{ClO}_4^-$  ratio in the Turpan-Hami samples, it would appear that there has not been much net addition of biogenic  $\text{NO}_3^-$  (Figure 2.3.8). Compared to the Mojave total deposition anion ratios and  $\text{NO}_3^-$  isotope values, the Turpan-Hami data are consistent with partial recycling of the atmospheric  $\text{NO}_3^-$  component. However given the extreme aridity and lack of vegetation, an alternative explanation may be that the Mojave total deposition  $\text{NO}_3^-/\text{ClO}_4^-$  ratios do not represent Turpan-Hami deposition ratios. For example, a lower contribution from wet deposition with higher  $\text{NO}_3^-/\text{ClO}_4^-$  might reduce the total deposition  $\text{NO}_3^-/\text{ClO}_4^-$  ratio and thus allow for biogenic addition without recycling. Similar to the Mojave, there also appears to be a substantial component of  $\text{Cl}^-$  unrelated to co-deposited  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  (Figure 2.3.7).

### ***Antarctica, University Valley***

$\text{ClO}_4^-$  concentrations varied over a relatively narrow range (50-500  $\mu\text{g/kg}$ ); 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  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  were correlated ( $r = 0.83 - 0.99$ ) in all profiles, as were concentrations of  $\text{Cl}^-$  with  $\text{ClO}_4^-$  ( $r = 0.72 - 0.99$ ) and  $\text{NO}_3^-$  ( $r = 0.64 - 0.99$ ) 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  $\text{NO}_3^-/\text{ClO}_4^-$ ,  $\text{Cl}/\text{ClO}_4^-$ , and  $\text{Cl}/\text{NO}_3^-$  respectively. The  $\text{NO}_3^-/\text{ClO}_4^-$  molar ratios were similar to those of Turpan-Hami samples, but an order of magnitude higher than Atacama ratios. The  $\text{Cl}/\text{NO}_3^-$  molar ratios for the Antarctica sites were lower than for all other sites, but the  $\text{Cl}/\text{ClO}_4^-$  ratios for the Atacama sites were lower than those for University Valley (Figure 2.3.7).

$\text{NO}_3^-$  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  $\delta^{18}\text{O}$  (>70‰) and  $\Delta^{17}\text{O}$  values (>29‰) (Michalski et al., 2005).  $\text{NO}_3^-$  in Antarctica soil samples analyzed in this study had a similar  $\delta^{18}\text{O}$  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  $0^\circ\text{C}$  as well as the limited precipitation.  $\text{NO}_3^-$  deposition in Antarctica is somewhat unique and  $\text{NO}_3^-$  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  $\text{NO}_3^-$  isotopic compositions that might reflect post-depositional transformations. The ratios of  $\text{NO}_3^-/\text{ClO}_4^-$  and  $\text{Cl}/\text{ClO}_4^-$ , and  $\text{NO}_3^-$  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***

$\text{ClO}_4^-$  concentrations ( $n=102$ ) in Atacama soil/caliche samples ranged from a minimum of  $0.5 \mu\text{g/kg}$  to a maximum of  $1 \times 10^6 \mu\text{g/kg}$  (Figure 2.3.5). The vast majority of profile samples (AT) collected in the central depression contained  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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,  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  were correlated except for the mine samples (Table 2.3.2). In general, correlation coefficients were lower for the relation between  $\text{Cl}^-$  and  $\text{ClO}_4^-$  compared to  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  but still generally significant, and as such  $\text{NO}_3^-$  and  $\text{Cl}^-$  were generally correlated.  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{NO}_3^-/\text{ClO}_4^- = 13,000$ ). All the Atacama  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  appears to be mainly biogenic (Figure 2.3.6).  $\text{Cl}^-/\text{ClO}_4^-$  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  $\text{NO}_3^-$  with only limited biogenic  $\text{NO}_3^-$  (Böhlke et al., 1997; Michalski et al., 2004). The average Atacama soil  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{ClO}_4^-$  at all other sites based on the stable isotopic composition of  $\text{ClO}_4^-$  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  $\text{NO}_3^-$  by denitrification or plant assimilation, given the hyper-arid conditions. The low ratio

could be due to regionally low  $\text{NO}_3^-$  deposition flux or high  $\text{ClO}_4^-$  deposition flux. Both of these species are considered to be at least partly atmospheric in origin based on elevated  $\Delta^{17}\text{O}$  values as well as  $^{36}\text{Cl}$  content of  $\text{ClO}_4^-$  (Bao and Gu, 2004; Michalski et al., 2004; Sturchio et al., 2009; Jackson et al., 2010). Regionally low  $\text{NO}_3^-$  production does not seem likely as the overall average  $\text{Cl}^-/\text{NO}_3^-$  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  $\text{ClO}_4^-$  presumably would require a mechanism to localize atmospheric production, as other sites in the southern hemisphere do not have such low  $\text{NO}_3^-/\text{ClO}_4^-$  ratios. Atacama  $\text{ClO}_4^-$  has a unique isotopic composition compared to other reported natural  $\text{ClO}_4^-$  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  $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ , and  $^{36}\text{Cl}/\text{Cl}$ ) could be related to an additional unknown production mechanism which could also explain the very low ratios of  $\text{NO}_3^-/\text{ClO}_4^-$ . Alternatively, given the duration of hyper-arid conditions in the Atacama ( $> 2$  million years), the relative enrichment and unique isotopic composition of  $\text{ClO}_4^-$  could be a reflection of atmospheric conditions that are no longer present. The age of the  $\text{ClO}_4^-$  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  $^{36}\text{Cl}/\text{Cl}$  ratios in  $\text{ClO}_4^-$  (Sturchio et al., 2009). This suggests that almost all of the Atacama  $\text{ClO}_4^-$  is older than 300,000 years, and thus predates the estimated time period over which  $\text{ClO}_4^-$  was deposited at other terrestrial locations.

#### 2.3.5.4 Implications of $\text{Cl}^-/\text{NO}_3^-/\text{ClO}_4^-$ Global Correlations

The global consistency of  $\text{NO}_3^-/\text{ClO}_4^-$  ratios in all locations other than the Atacama is in contrast to the more variable  $\text{Cl}^-/\text{NO}_3^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  ratios (Figure 2.3.7).  $\text{Cl}^-/\text{NO}_3^-$  ratios are commonly used to evaluate N and/or  $\text{NO}_3^-$  gains/losses in various ecosystems. Co-variation of  $\text{Cl}^-/\text{NO}_3^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  ratios illustrated in Figure 2.3.7 indicates large variations in  $\text{Cl}^-$  are related to processes that either (1) do not affect  $\text{NO}_3^-$  or  $\text{ClO}_4^-$ , or (2) affect both  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{NO}_3^-/\text{ClO}_4^-$  ratios similar to atmospheric deposition ratios, whereas  $\text{Cl}^-/\text{NO}_3^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  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  $\text{Cl}^-/\text{NO}_3^-$  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 ( $\text{Cl}^-/\text{NO}_3^- = 0.27$ ) (Plummer et al., 2006) as well as that calculated using reported data (2000-2010) for total deposition from 95 sites across the U.S. ( $\text{Cl}^-/\text{NO}_3^- = 0.29$ ;  $n=892$ ) (CASTNET, 2014).

$\text{Cl}^-$  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  $\text{Cl}^-/\text{NO}_3^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  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  $\text{Cl}^-$  deposition. Similarly, continental-scale studies of  $^{36}\text{Cl}/\text{Cl}$  ratios in pre-anthropogenic groundwater in the U.S. and modern atmospheric deposition in Europe indicated maximum coastal marine  $\text{Cl}^-$  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  $\text{Cl}^-$ . The  $\text{Cl}^-/\text{NO}_3^-$  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  $\text{Cl}^-/\text{NO}_3^-$  ratios are from areas near coasts (e.g., Namibia and UAE). The highest wet deposition  $\text{Cl}^-/\text{NO}_3^-$  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  $\text{Cl}^-/\text{NO}_3^-$  and  $\text{Cl}^-/\text{ClO}_4^-$  ratios (Figure 2.3.7), and the lack of a relation between the increase in  $\text{Cl}^-/\text{NO}_3^-$  ratio and the  $\text{NO}_3^-/\text{ClO}_4^-$  ratio (Figure 2.3.7) suggests that either  $\text{Cl}^-$  at most sites has been supplemented by sources unrelated to the source of the original co-deposited  $\text{NO}_3^-$  or  $\text{ClO}_4^-$ , or that both  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  were equally lost in these systems as previously described. Partial microbial reduction of  $\text{NO}_3^-$  is supported by isotope data at some sites (Figure 2.3.6). However,  $\text{NO}_3^-$  isotopes at most sites appear to be relatively unfractionated and some indicate substantial unaltered atmospheric  $\text{NO}_3^-$ . For  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  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  $\text{Cl}^-/\text{NO}_3^-$  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  $\text{Cl}^-$  concentrations contain atmospheric  $\text{Cl}^-$  that accumulated during previous wetter periods that supported biological reduction of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  (non-co-deposited  $\text{Cl}^-$ ).

Another possible confounding factor may be the potential de-coupling of  $\text{Cl}^-$  and  $\text{NO}_3^-$  or  $\text{ClO}_4^-$  transport. Local redistribution of salts by selective crystallization, dissolution, and infiltration might have separated  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  from  $\text{Cl}^-$  at the scale of individual samples or vertical profiles. Also, we have observed that at least some plants uptake  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  proportionally while excluding  $\text{Cl}^-$  (Andraski et al., 2014). Biologic assimilation could be responsible for substantial N sequestration in persistent organic matter, and release of N gases (e.g.,  $\text{NH}_4$ ,  $\text{N}_2\text{O}$ ,  $\text{N}_2$ ) during remineralization, nitrification, or denitrification, could also lead to elevated  $\text{Cl}^-/\text{NO}_3^-$  ratios in soils and recharging groundwaters, but existing data do not support similar losses of  $\text{ClO}_4^-$  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

$\text{ClO}_4^-$  is globally distributed in soil and groundwater in arid and semi-arid regions on Earth at concentrations ranging from  $10^{-1}$  to  $10^6$   $\mu\text{g/kg}$ . Generally, the  $\text{ClO}_4^-$  concentration in these regions increases with aridity index, but also depends on the duration of arid conditions. In many arid and semi-arid areas,  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  co-occur at consistent ratios ( $\text{NO}_3^-/\text{ClO}_4^-$ ) 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  $\text{N}_2$  fixation, N mineralization, nitrification, denitrification, and microbial  $\text{ClO}_4^-$  reduction. At first approximation, relatively constant  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{Cl}^-/\text{ClO}_4^-$  and  $\text{Cl}^-/\text{NO}_3^-$  ratios indicate  $\text{Cl}^-$  varies independently from both  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ . This is likely due to variation of sea salt  $\text{Cl}^-$  in deposition, plus variable addition of  $\text{Cl}^-$  from dust or subsurface sedimentary and evaporitic sources, and possibly excess  $\text{Cl}^-$  accumulation during past wetter periods of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  reduction. The general lack of correlation between  $\text{Cl}^-$  and  $\text{ClO}_4^-$  or  $\text{NO}_3^-$  implies that  $\text{Cl}^-$  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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  in arid and semi-arid locations, and associated variations in the isotopic composition of the  $\text{NO}_3^-$ , are consistent with a conceptual model of atmospheric origin, global co-deposition, and variable alteration of the  $\text{NO}_3^-$  pool by biogenic addition, assimilation, and/or recycling on the surface. Preservation of the atmospheric deposition  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{ClO}_4^-$  concentration in desert regions could be useful for interpreting major anion cycles and processes, particularly with respect to the less-conserved  $\text{NO}_3^-$  pool. If more accurate local measures of deposition can be obtained, then  $\text{ClO}_4^-$  surface accumulations in conjunction with  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  compared to  $\text{Cl}^-$  or  $\text{NO}_3^-$ , accompanied by unique  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  production rates, or higher  $\text{ClO}_4^-$  production rates in pre-Pleistocene times.

In the absence of microbial reduction,  $\text{ClO}_4^-$  can persist in many environmental conditions. On early Earth, prior to the onset of microbial  $\text{ClO}_4^-$  reduction,  $\text{ClO}_4^-$  concentrations could have been much higher than currently observed. However, the net impact of microbial  $\text{ClO}_4^-$  reduction on the global chlorine biogeochemical cycle remains unconstrained.

Elevated concentrations of  $\text{ClO}_4^-$  reported on the surface of Mars, and its enrichment with respect to  $\text{Cl}^-$  and  $\text{NO}_3^-$ , could reveal important clues regarding the climatic, hydrologic, and potentially biologic evolution of the planet. Given the highly conserved ratio of  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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  $0^\circ\text{C}$ , 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  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$ . The purpose of this task was to measure  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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**

$\text{ClO}_4^-$ ,  $\text{ClO}_3^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{Cl}^-$  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.lternet.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 ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ , and  $\text{SO}_4^{2-}$ ) 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  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  concentrations with respect to other anions on a consistent set of samples.  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$ , 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  $\pm 10\%$  and the spike recoveries were between 90 and 110 %. Samples with elevated  $\text{Cl}^-$  ( $>10,000$  mg/L) or  $\text{SO}_4^{2-}$  ( $>1000$  mg/L) were either diluted prior to analysis or in some cases pre-cleaned using On-Guard™ II Ag or Ba cartridges (Dionex).

### 2.4.4 Modeling

Two simple models were used to evaluate  $\text{ClO}_4^-$ ,  $\text{ClO}_3^-$ , and  $\text{SO}_4^{2-}$  concentrations relative to the conserved species  $\text{Cl}^-$  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  $\text{Cl}^-$ . For each species we calculated the mass ratio with respect to  $\text{Cl}^-$  (e.g.  $\text{ClO}_4^-/\text{Cl}^-$ ) at the depth closest to the bottom of the lake ice. This ratio was then multiplied by the  $\text{Cl}^-$  concentration at lower depths to determine the expected concentration of each species as shown below.

$$C_{Dx} = C_{Ds}/\text{Cl}^-_{Ds} \times \text{Cl}^-_{Dx}$$

Where  $C_{Dx}$  and  $C_{Ds}$  are the concentration of  $\text{ClO}_4^-$ ,  $\text{ClO}_3^-$ , or  $\text{SO}_4^{2-}$  at depth X and the surface, respectively; and  $\text{Cl}^-_{Ds}$  and  $\text{Cl}^-_{Dx}$  are the concentrations of  $\text{Cl}^-$  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  $\text{Cl}^-$  profile using a simple mass balance

$$\text{Cl}^-_{Dx} = f^s_{Dx} \text{Cl}^-_{Ds} + f^{35}_{Dx} \text{Cl}^-_{35}$$

where  $f^s_{Dx}$  and  $f^{35}_{Dx}$  are the fraction of surface water and water at 35m respectively at any depth  $D_x$ ,  $\text{Cl}^-_{35}$  is the concentration at 35m and all other variables are previously defined. The values of  $f^s_{Dx}$  and  $f^{35}_{Dx}$  determined for each depth using  $\text{Cl}^-$  were then used to calculate the concentration of

$\text{ClO}_4^-$ ,  $\text{ClO}_3^-$ , or  $\text{SO}_4^{2-}$  using the same mass balance but substituting the concentrations of each species for  $\text{Cl}^-$  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  $\text{Cl}^-$ . 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).  $\text{ClO}_4^-$  concentrations in a subset of these water bodies ranged from 0.05-8.1  $\mu\text{g/l}$  but were generally less than 0.5 (45/49 samples) with an overall average of 0.19  $\mu\text{g/l}$  excluding one outlier (Parera Pond) (Table 1). Of the samples that exceeded 0.5  $\mu\text{g/l}$  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 (0.05 $\mu\text{g/l}$ ). 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  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  concentrations in surface waters of the MDV are higher than those reported for wet precipitation from North America (average = 0.014  $\mu\text{g/L}$ ) (Rajagopalan et al., 2009) or in ice cores from Wyoming ( $0.0002 < 0.009 \mu\text{g/L}$ ), Yukon Territory ( $0.0002 < - 0.002 \mu\text{g/L}$ ), and the Devon Ice Cap ( $0.001\text{-}0.015 \mu\text{g/L}$ ) (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.  $\text{ClO}_3^-$  concentrations in the non-lake surface water bodies were similar to  $\text{ClO}_4^-$  with concentrations ranging from  $<0.05$  to 15.5  $\mu\text{g/l}$  and an overall average excluding Goldman Pond of 0.25  $\mu\text{g/l}$  (Table 2.4.1). Little information is available concerning  $\text{ClO}_3^-$  in the environment but ratios of  $\text{ClO}_3^-/\text{ClO}_4^-$  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  $3.8 \pm 2.9$  (Furdui and Tomassini, 2010). Samples from Taylor and Wright Valleys have an average ratio (W/W) of  $1.7 \pm 1.1$  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  $\text{Cl}^-$  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 oxidic (~20 mg  $\text{O}_2/\text{l}$ ) 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  $\text{NO}_3^-$  is below detection ( $<0.5 \mu\text{g N/l}$ ) above 15m and increases due to nitrification below 15m reaching a maximum of  $\sim 112 \mu\text{gN/l}$  (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 ( $\sim 0.7 \text{ mg/l}$ ) of  $\text{H}_2\text{S}$  near the sediment-water interface clearly indicates anoxic processes are occurring near the sediment water interface (Clocksin et al., 2007).

Our results show that  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  in Lake Hoare are present throughout the water column at concentrations ranging from 0.1 to 0.3 and 0.15 to  $0.42 \mu\text{g/l}$ , 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,  $\text{Cl}^-$  concentrations decrease exponentially towards the surface.  $\text{SO}_4^{2-}$  follows a similar trend increasing proportionally to  $\text{Cl}^-$  with the exception of the deepest sample (30m) consistent with the presence of  $\text{H}_2\text{S}$  at the same depth. Neither  $\text{ClO}_4^-$  nor  $\text{ClO}_3^-$  concentrations increase proportionally to  $\text{Cl}^-$ , although the concentrations do initially increase.

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

The concentration of  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  can likely be explained by a similar process as  $\text{SO}_4^{2-}$ . Like  $\text{NO}_3^-$  both  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  are only reduced at oxygen concentrations below  $\sim 1 \text{ mg/l}$ . Therefore given the elevated  $\text{O}_2$  concentrations throughout the lake depth,  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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  $\text{ClO}_x^-$  reduction. Both  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  are utilized as electron acceptors prior to  $\text{SO}_4^{2-}$ , and consequently the upper portions of the sediment should support  $\text{ClO}_x^-$  reduction. The very large differences in concentrations of  $\text{SO}_4^{2-}$  and  $\text{ClO}_x^-$  ( $\text{SO}_4^{2-}/\text{ClO}_x^- > 10,000$ ) explain the difference in  $\text{ClO}_x^-$  depth profiles compared to  $\text{SO}_4^{2-}$ . The relatively low organic matter input to the sediments sustains relatively low rates of electron acceptor ( $\text{O}_2$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ) reduction, but given the ratio of  $\text{SO}_4^{2-}$  to  $\text{ClO}_x^-$  this rate would lead to the rapid consumption of  $\text{ClO}_x^-$  but a negligible decrease in  $\text{SO}_4^{2-}$  concentration. This would eventually

lead to a depleted  $\text{ClO}_x^-$  concentration profile relative to  $\text{Cl}^-$  throughout most of the depth of the lake as observed. Hence, one implication of the data presented here is that  $\text{ClO}_x$  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  $\text{NO}_3^-$  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 ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ) 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  $\text{N}_2\text{O}$  in EL increases similarly below the chemocline and declines rapidly (<1uM) below 30m. The anomalously elevated concentrations of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  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  $^{15}\text{N}$  and  $^{18}\text{O}$  in  $\text{N}_2\text{O}$  in concert with genomic studies suggest the origin of the  $\text{N}_2\text{O}$  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  $\text{SO}_4^{2-}/\text{Cl}^-$  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  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , as well as the lack of any  $\text{H}_2\text{S}$ , the

reduction in ratio is most likely due to precipitation of a  $\text{SO}_4^{2-}$  mineral phase in the hypersaline waters rather than sulfate reduction.

$\text{ClO}_4^-$  concentration increases with depth in the EL of Lake Bonney (0.46 to 8.3  $\mu\text{g/l}$  at the surface and 35m, respectively) (Figure 2.4.4). The increase in  $\text{ClO}_4^-$  concentration is proportional to the increase in  $\text{Cl}^-$  concentration ( $r^2=0.99$ ) over the entire water column.  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  concentrations are predicted accurately with a simple mixing model of concentrations at the surface and 35m (Dashed lines Figure 2.4.4). The  $\text{ClO}_4^-/\text{Cl}^-$  molar ratio ( $5 \times 10^{-7}$ ) at the shallowest depth (4m) is less than the ratio in Taylor Valley surface streams ( $4 \times 10^{-6}$ ) and decreases with depth to a relatively constant value of  $\sim 6 \times 10^{-9}$  at 35m (Figure 2.4.3). The ratio at 35 m is still substantially enriched compared to seawater  $3.0 \times 10^{-10}$  which is considered the major source of salts in the EL. It is possible that water below 18m became enriched in  $\text{ClO}_4^-$  relative to  $\text{Cl}^-$  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).  $\text{Cl}^-$  concentrations reach a maximum at 24m and remain constant at lower depths (Lyons et al., 2005).  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  at depth with respect to  $\text{Cl}^-$ ) of biological reduction of  $\text{ClO}_4^-$  which is not unexpected given the stability of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  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  $\text{ClO}_4^-$  concentration profiles and  $\text{ClO}_4^-/\text{Cl}^-$  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  $\text{ClO}_4^-$  in the EL could be a mix of surface water inflow and seawater, although it is currently not possible to determine if the  $\text{ClO}_4^-$  in the original seawater is still present. It is possible the east lobe could have experienced conditions that supported  $\text{ClO}_4^-$  reduction in the distant past given the estimated age of isolation of the seawater 1.7-5.1Ma. Regardless, the  $\text{ClO}_4^-$  must be reasonably old as the concentration at 35 m is at least  $\sim 70\times$  the average Taylor Valley stream concentration and  $170\times$  seawater concentrations.

**Table 2.4.1.  $\text{ClO}_3^-$ ,  $\text{ClO}_4^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  concentrations in surface waters of the Dry Valleys.**

| Valley        | Basin     | Water Body     | Date    | $\text{ClO}_4^-$ | $\text{ClO}_3^-$ | $\text{Cl}^-$ | $\text{SO}_4^{2-}$ |
|---------------|-----------|----------------|---------|------------------|------------------|---------------|--------------------|
|               |           |                |         | $\mu\text{g/L}$  |                  | $\text{mg/l}$ |                    |
| Taylor Valley | E. Bonney | Priscu         | 12/2009 | 0.07             | 0.20             | 10.7          | 5.4                |
|               |           | Priscu         | 12/2008 | 0.15             | 0.35             | 9.8           | 6.3                |
|               |           | Priscu         | 01/2009 | 0.47             | 0.47             | 43.0          | 11.3               |
|               |           |                |         |                  |                  |               |                    |
|               | W. Bonney | Lawson         | 01/2009 | 0.14             | 0.18             | 8.22          | 5.4                |
|               |           | Lawson         | 12/2008 | 0.25             | 0.17             | 5.7           | 3.6                |
|               |           | Lawson         | 12/2009 | 0.18             | 0.25             | 4.9           | 3.5                |
|               |           | Blood Falls    | 12/2008 | < 0.05           | < 0.05           |               |                    |
|               |           |                |         |                  |                  |               |                    |
|               | Fryxell   | Aiken          | 12/2009 | 0.18             | 0.21             | 12.8          | 3.6                |
|               |           | Aiken          | 12/2009 | 0.12             | < 0.1            | 7.1           | 3.2                |
|               |           | Canada         | 01/2009 | 0.06             | .55              | 2.4           | 2.4                |
|               |           | Canada         | 12/2009 | 0.09             | <0.1             | 2.2           | 3.3                |
|               |           | Canada         | 01/2010 | 0.06             | 0.25             | 1.7           | 2.2                |
|               |           | Canada         | 12/2008 | 0.13             | 0.11             | 4.0           | 3.9                |
|               |           | Common Wealth* | 12/2008 | 0.10             | 0.21             | 4.7           | 2.2                |
|               |           | Crescent       | 12/2008 | 0.51             | 0.48             | 14.1          | 6.6                |
|               |           | Delta          | 01/2009 | 0.34             | 0.25             | 9.1           | 6.0                |
|               |           | Delta          | 01/2010 | 0.16             | 0.11             | 15.4          | 6.8                |
|               |           | Delta          | 12/2008 | 0.19             | 0.22             | 18.9          | 6.4                |
|               |           | Fryxell Pond   | 01/2010 | 0.13             | 0.48             | 8.4           | 3.3                |
|               |           | Green          | 01/2009 | < 0.05           | < 0.05           | 4.0           | 0.6                |
|               |           | Green          | 01/2010 | 0.09             | 0.35             | 1.4           | 0.7                |
|               |           | Green          | 12/2008 | 0.06             | < 0.1            | 2.3           | 0.9                |
|               |           | Green          | 01/2009 | 0.05             | 0.15             | 2.7           | 0.9                |
|               |           | Harnish        | 01/2009 | 0.7              | 0.22             | 13.7          | 7.8                |
|               |           | Howard Pond    | 01/2010 | 0.11             | 0.20             | 8.0           | 3.9                |
|               |           | Huey           | 01/2009 | 0.21             | 0.26             | 10.3          | 12.3               |
|               |           | Huey           | 12/2008 | 0.43             | 0.43             | 10.3          | 12.1               |
|               |           | Lost Seal      | 12/2008 | 0.18             | 0.07             | 12.6          | 5.4                |
|               |           | Lost Seal      | 01/2010 | 0.07             | <0.1             | 7.9           | 3.6                |
|               |           | Lost Seal      | 12/2008 | 0.51             | 0.24             | 12.7          | 5.9                |
|               |           | Von Guerard    | 01/2009 | 0.16             | 0.05             | 6.1           | 3.2                |
|               |           | Von Guerard    | 12/2008 | 0.49             | < 0.1            | 6.8           | 3.8                |
|               |           | Von Guerard    | 01/2010 | 0.01             | <0.1             | 61.7          | 13.7               |
|               |           |                |         |                  |                  |               |                    |
|               | Hoare     | Andersen       | 12/2008 | 0.1              | <0.05            | 2.8           | 3.8                |
|               |           | Andersen       | 12/2009 | 0.09             | <0.1             | 1.9           | 2.2                |
|               |           | Andersen       | 12/2008 | 0.07             | <0.1             | 3.1           | 5.4                |
|               |           | Andersen       | 12/2008 | 0.18             | 0.17             | 14.2          | 11.1               |
|               |           | House          | 12/2008 | 0.10             | <0.05            | 3.1           | 2.7                |
|               |           | House          | 12/2008 | 0.13             | 0.11             | 3.6           | 3.5                |
|               |           | House          | 12/2008 | 0.09             | 0.21             | 6.6           | 3.1                |
|               |           | House          | 12/2009 | 0.08             | <0.1             | 2.4           | 2.3                |
|               |           | Wharton        | 12/2009 | 0.11             | <0.1             | 2.0           | 1.6                |
|               |           |                |         |                  |                  |               |                    |
|               |           | Parera Pond    | 01/2010 | 8.1              | 15.5             | 1300          | 198                |
| Wright        | Vanda     | Onyx           | 12/2009 | 0.08             | 0.39             | 5.2           | 2.5                |
|               |           | Onyx           | 11/2009 | 0.13             | 0.16             | 6.8           | 3.4                |
|               |           | Onyx           | 01/2009 | 0.08             | 0.43             | 4.9           | 2.6                |
|               |           | Onyx           | 01/2009 | 0.13             | 0.28             | 8.0           | 4.8                |
|               |           | Onyx           | 12/2008 | 0.06             | <0.1             | 8.9           | 2.7                |
|               |           | Onyx           | 12/2008 | 0.20             | 0.34             | 7.9           | 5.1                |
|               |           | Onyx           | 01/2010 | 0.17             | 0.18             | 5.9           | 3.9                |
|               |           |                |         |                  |                  |               |                    |

\*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.



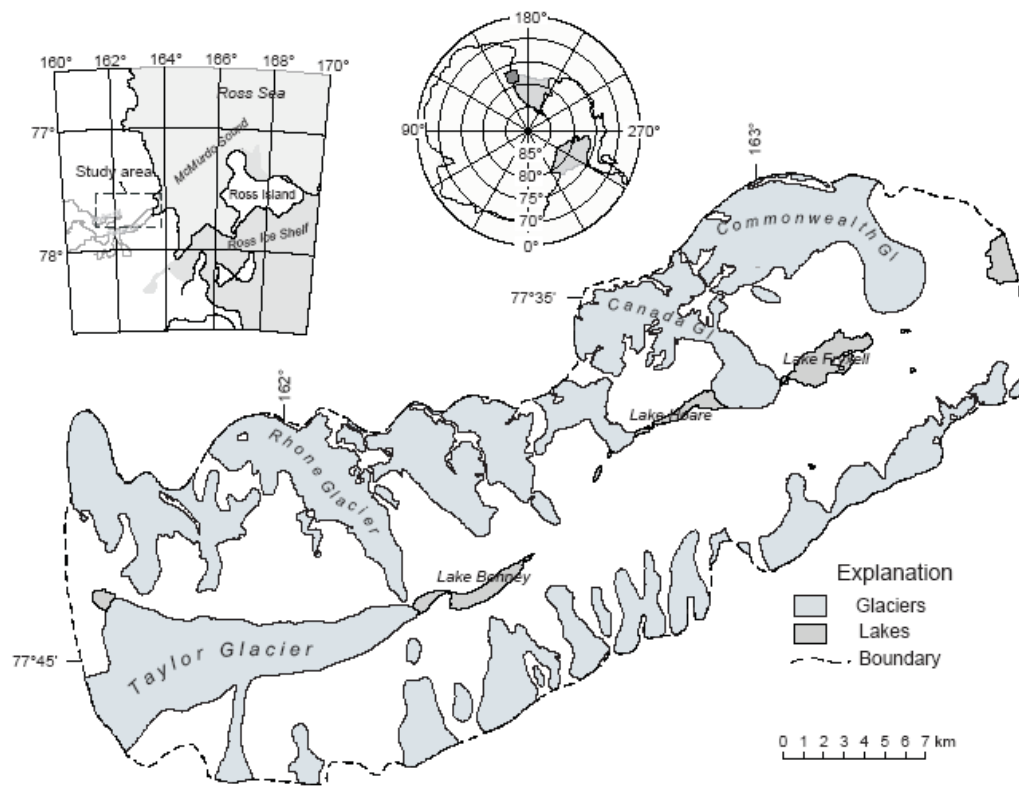


**Figure 2.4.1. Map of Taylor Valley, Antarctica showing the locations of Lake Fryxell, Lake Bonney, and Lake Hoare. Adapted from Lyons et al., (2005).**

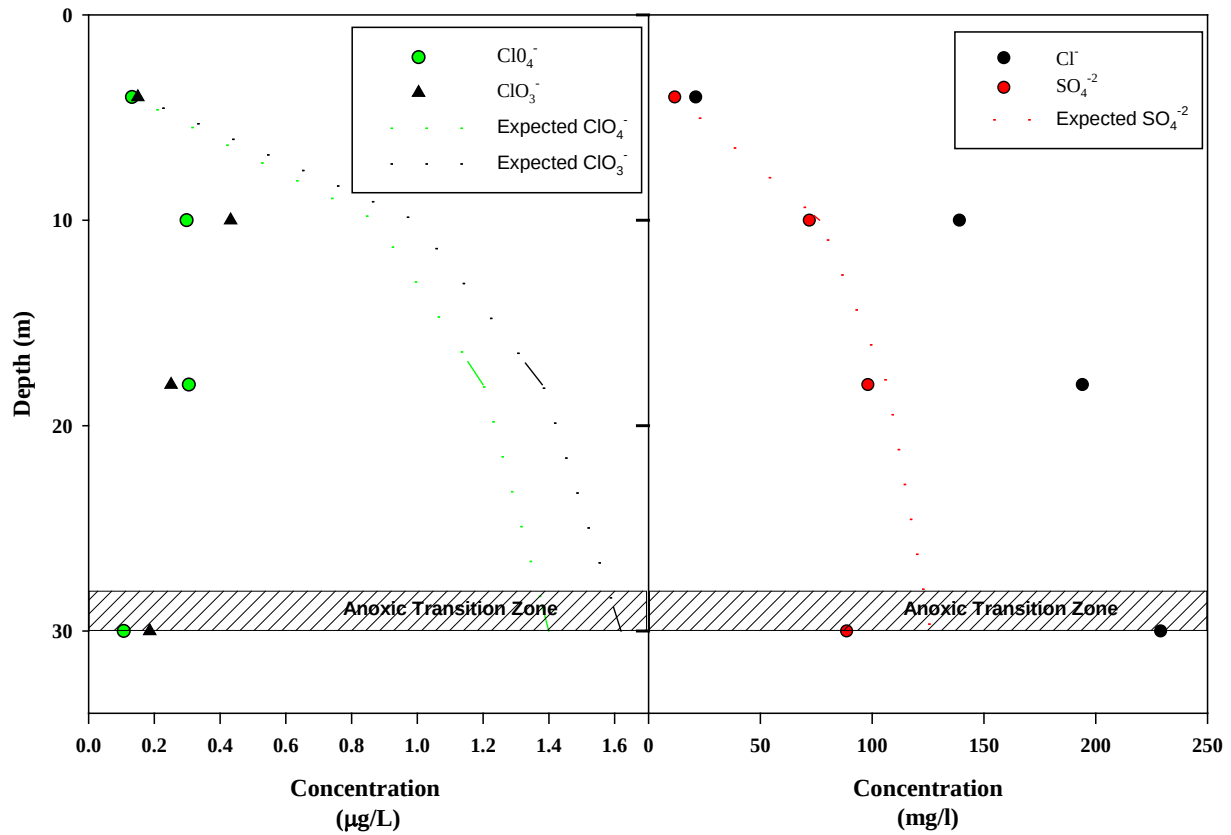


**Figure 2.4.2. Concentration profiles of  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  in Lake Hoare, December 2009.** Dashed lines represent modeled concentrations based on maintaining surface water ratios of  $\text{ClO}_3^-$ ,  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  relative to  $\text{Cl}^-$  throughout the lake depth.

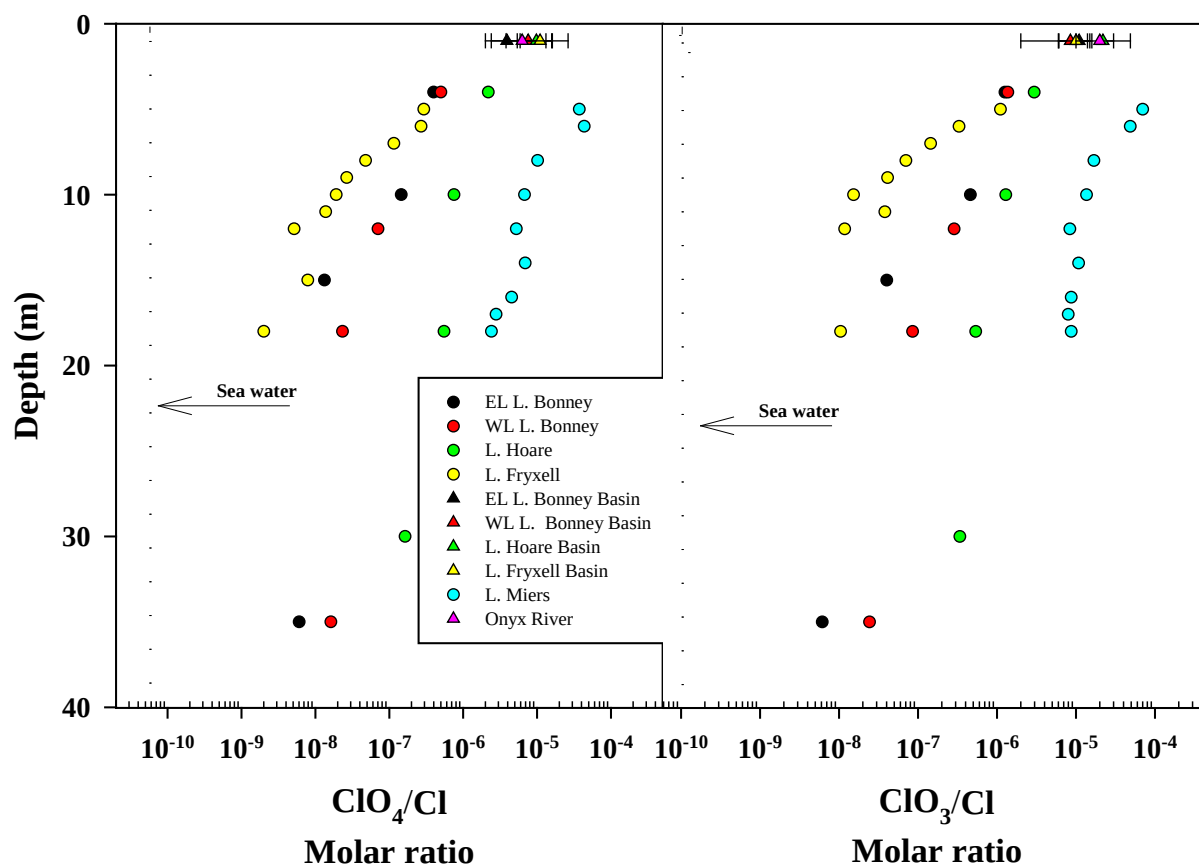


**Figure 2.4.3. Molar ratios of  $\text{ClO}_4^-/\text{Cl}^-$  and  $\text{ClO}_3^-/\text{Cl}^-$  with depth in MCM lakes and feed waters for lake basins in Taylor Valley and the Onyx River in Wright Valley. The black dashed line in each figure represents the ratio in seawater sampled at McMurdo Station.**

In contrast to  $\text{ClO}_4^-$ ,  $\text{ClO}_3^-$  is not as conservative with respect to  $\text{Cl}^-$  concentration.  $\text{ClO}_3^-$  concentration does increase with depth (1.1 and 10.5  $\mu\text{g/l}$  at 4m and 35m, respectively) but the increase is proportional to  $\text{Cl}^-$  only to a depth of 18m (Figure 2.4.4). This is highlighted by evaluating the relationship between  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  (Figure 2.4.5) which is highly correlated ( $R=0.97$ ) up to a depth of 18m. The predicted  $\text{ClO}_3^-/\text{ClO}_4^-$  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  $\text{ClO}_3^-$  relative to  $\text{ClO}_4^-$ . Modeled  $\text{ClO}_3^-$  concentrations at 4, 12, and 18m are well predicted based on the two parts mixing model and using the predicted  $\text{ClO}_3^-$  concentration at 35m based on the  $\text{ClO}_4^-$  concentration.  $\text{ClO}_3^-/\text{Cl}^-$  molar ratios in EL surface water ( $1 \times 10^{-6}$ ) are similar to streams throughout Taylor Valley and are still well above seawater ratios ( $1 \times 10^{-10}$ ) even with the apparent reduction of  $\text{ClO}_3^-$  at 35m. What is clear is that at least at 35m some loss of  $\text{ClO}_3^-$  relative to  $\text{ClO}_4^-$  has occurred. This could be due to low level  $\text{ClO}_3^-$  reduction by low level nitrate reductase activity. While  $\text{NO}_3^-$  and  $\text{NO}_2^-$  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  $\text{ClO}_3^-$  has been reduced.  $\text{ClO}_3^-$  concentrations are only 0.1 and 0.3% of the molar concentrations of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , respectively and therefore reduction rates too small to impact bulk  $\text{NO}_3^-$  and  $\text{NO}_2^-$  could have an impact on  $\text{ClO}_3^-$  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.  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{N}_2\text{O}$  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  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  are similar to the EL except at the lowest sampling depth (35m) at which concentrations are ~5 and 8X lower even though  $\text{Cl}^-$  is only 2X lower

(Figure 2.4.6).  $\text{ClO}_4^-/\text{Cl}^-$  and  $\text{ClO}_3^-/\text{Cl}^-$  ratios are also similar for comparable depths (Figure 2.4.3). The loss of  $\text{ClO}_3^-$  relative to  $\text{ClO}_4^-$  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  $\text{SO}_4^{2-}$  and  $\text{ClO}_4^-$ , although some loss of  $\text{ClO}_4^-$  has occurred at 35m. Predicted concentrations of  $\text{ClO}_3^-$  are similar for depths above 35m if the concentration at 35m is predicted from the  $\text{ClO}_4^-$  concentration and expected ratio of  $\text{ClO}_3^-/\text{ClO}_4^-$  from Figure 2.4.5.  $\text{ClO}_4^-$  concentration at 35m and the ratio from Figure 2.4.5 is used to predict the  $\text{ClO}_3^-$  concentration at 35m rather than the measured concentration. The differences between the EL and WL are likely due to the biological reduction of  $\text{ClO}_4^-$ ,  $\text{ClO}_3^-$  and  $\text{NO}_3^-$  at depth in the WL as opposed to only  $\text{ClO}_3^-$  in the EL. The WL has an active denitrification zone below 18m and  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{N}_2\text{O}$  are completely reduced by 30m (Voytek et al., 1999; Priscu et al., 1996, Priscu 1997). Taken together this implies that loss of  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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  $\text{ClO}_4^-$  which is only slightly depleted with respect to  $\text{Cl}^-$ .

### ***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 oxicleine (~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  $\text{Cl}^-$  profile steadily increases with depth and has previously been attributed to diffusion of  $\text{Cl}^-$  from the sediment to the surface (Green and Lyons, 2009). At depths above ~9m the water is supersaturated with  $\text{O}_2$  and between ~9-11m a chemocline exists in which  $\text{O}_2$  is rapidly depleted and in which chlorophyll-a reaches a concentration maximum (Priscu, 1995).  $\text{SO}_4^{2-}$  slowly increases with depth to a maximum at ~11-12m and then rapidly decreases to below 0.1mM at the sediment water interface.  $\text{H}_2\text{S}$  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  $\text{Cl}^-$  with a minimum at the surface (~3.0 mg/l) and a maximum (31.2 mg/l) at the sediment water interface. Like  $\text{Cl}^-$  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).  $\text{NH}_4^+$  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.  $\text{NO}_3^-$  was below detection for all depths and  $\text{NO}_2^-$  was present at trace concentrations (<0.0042 mg-N/L) at 7m and below 15m (Voytek et al., 1999).

Concentrations of both  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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  $\text{ClO}_4^-/\text{Cl}^-$  and  $\text{ClO}_3^-/\text{Cl}^-$  are the lowest of all the lakes, likely due to both the addition of  $\text{Cl}^-$  from the sediment and strongly reducing conditions below 11m that facilitate  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  bacterial reduction. Predicted concentrations based on surface water ratios of  $\text{ClO}_4^-/\text{Cl}^-$  and  $\text{ClO}_3^-/\text{Cl}^-$  over estimate both  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  concentrations at depths below 6m.  $\text{SO}_4^{2-}$  concentrations are likewise over predicted at depths below the chemocline. Collectively, these results in concert with the complete lack of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  below the chemocline (Priscu and Ward, 1999, Priscu 1997) suggests that  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  are actively being reduced. These data further imply that  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  were either not present during the last lake drawdown ( $\sim 1000\text{Ka}$ ) or were consumed since the lake has been refilled. The  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  concentration profile appear to reflect a surface source, distribution by diffusion, and consumption at depth below 11m.

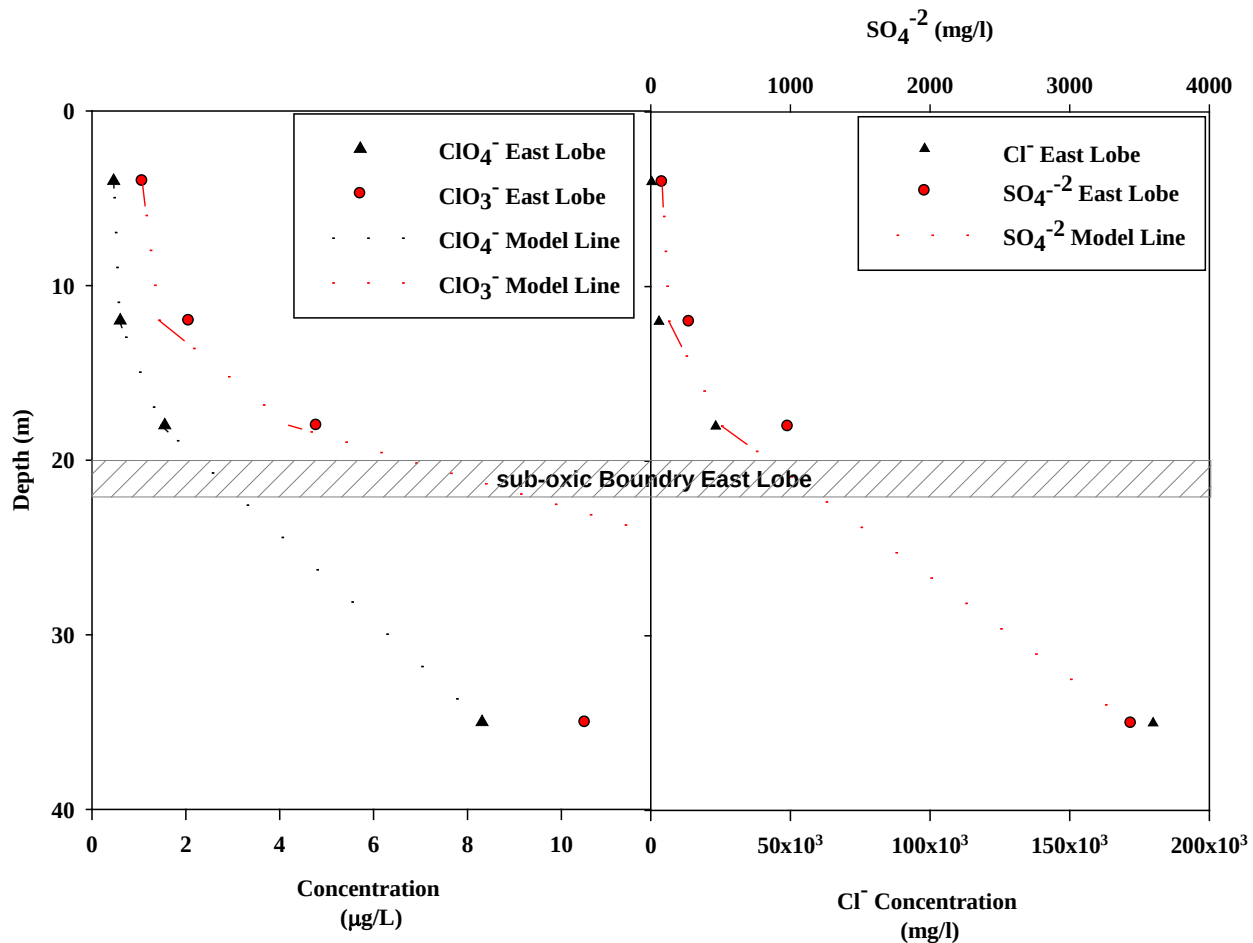


**Figure 2.4.4. Concentration profiles of  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  in the EL of Lake Bonney, December 2009.** Dashed lines represent modeled concentrations based on a two part mixing model of concentrations at the surface and 35m except for  $\text{ClO}_3^-$  for which the concentration at 35m is predicted from the  $\text{ClO}_4^-$  concentration using the ratio from Figure 2.4.5. See text for further explanation.

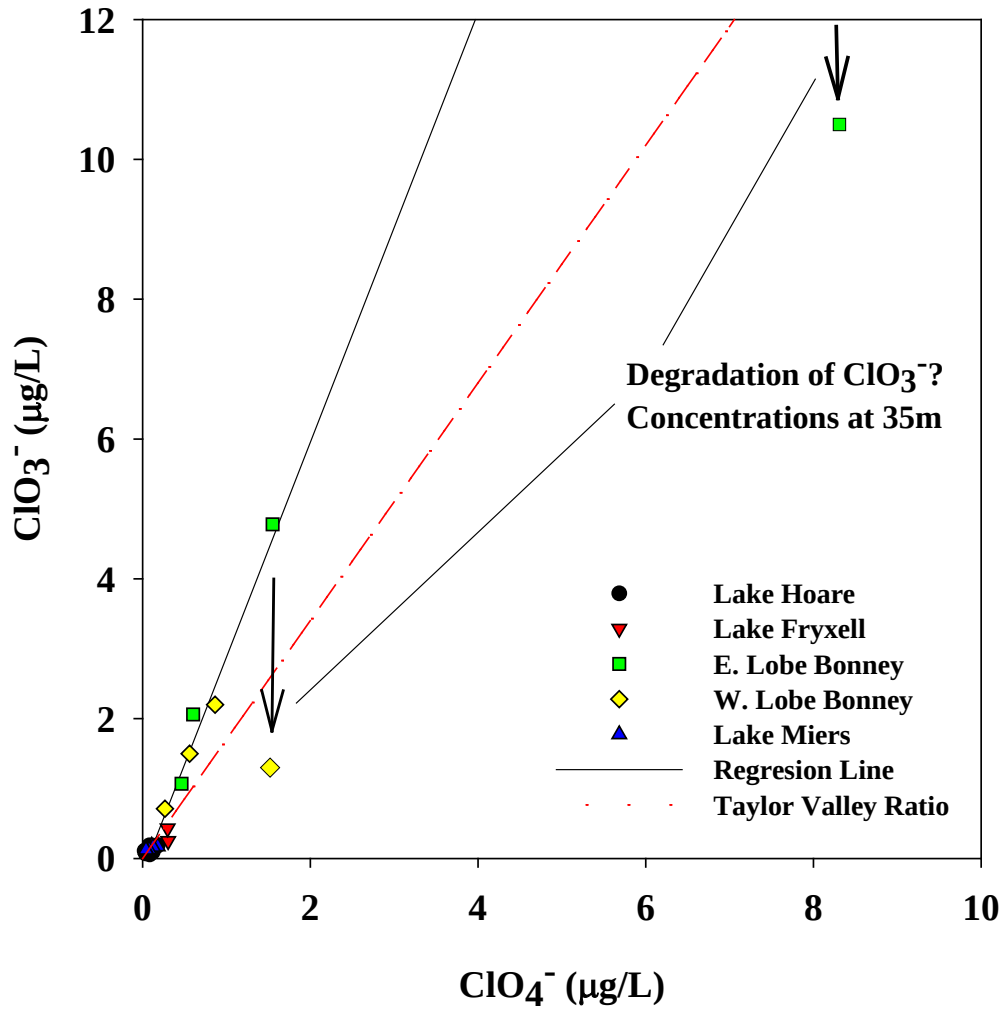


**Figure 2.4.5. Relationship of ClO<sub>3</sub><sup>-</sup> and ClO<sub>4</sub><sup>-</sup> concentration in Dry Valley Lakes.** The solid black line represents the regression line ( $r^2 = 0.97$  and ratio = 3.1) and the red dashed line represents ratio of ClO<sub>3</sub><sup>-</sup> and ClO<sub>4</sub><sup>-</sup> in streams of Taylor Valley.



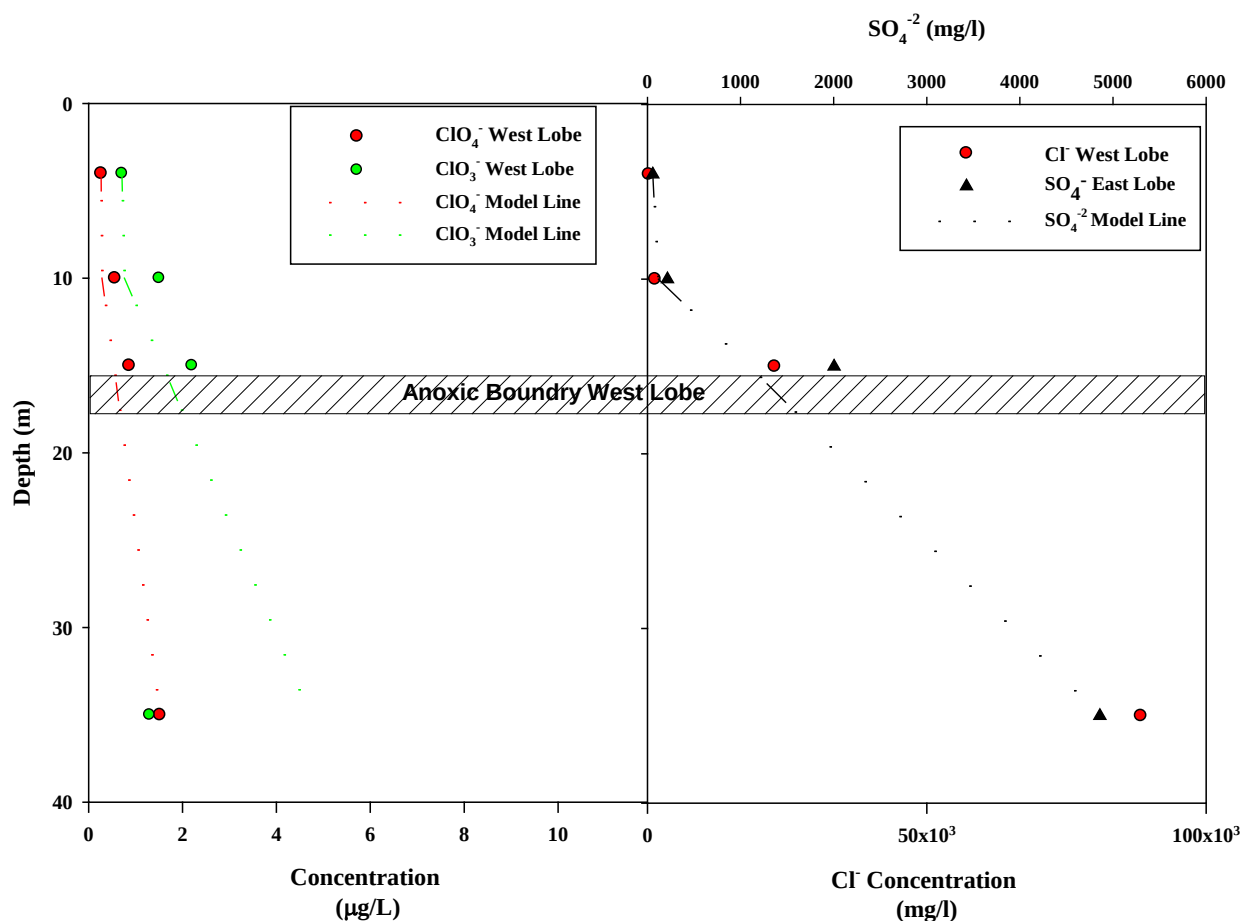


**Figure 2.4.6. Concentration profiles of  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  in the WL of Lake Bonney, December 2009.** Dashed lines represent modeled concentrations based on a two part mixing model of concentrations at the surface and 35m except for  $\text{ClO}_3^-$  for which the concentration at 35m is predicted from the  $\text{ClO}_4^-$  concentration using the ratio from Figure 2.4.5. See text for more explanation.

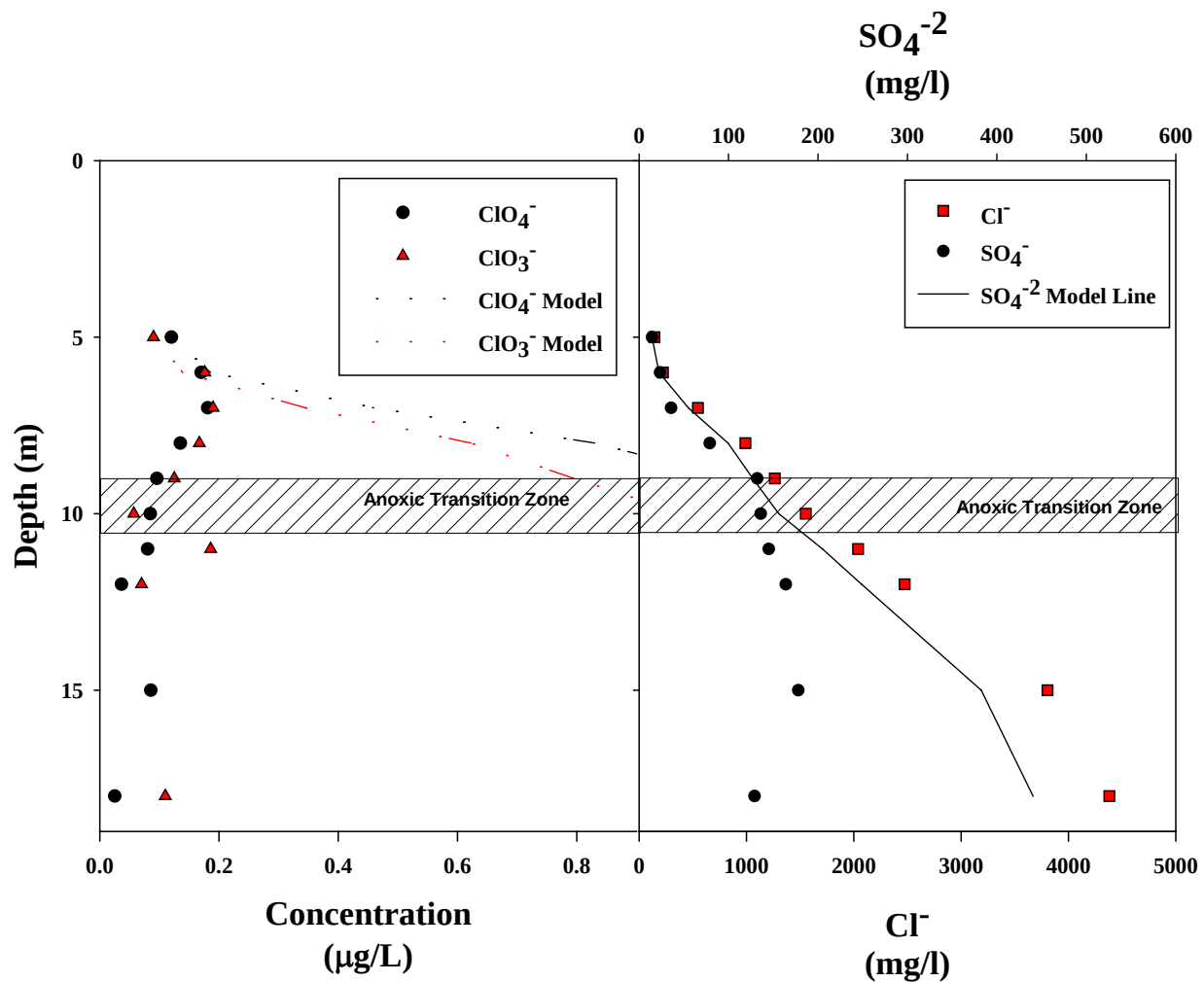


**Figure 2.4.7. Concentration profiles of  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  in Lake Fryxell, December 2008.** Dashed lines represent modeled concentrations based on maintaining surface water ratios of  $\text{ClO}_3^-$ ,  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  relative to  $\text{Cl}^-$  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  $\text{NO}_3^-$  and  $\text{NO}_2^-$  are essentially constant with depth (<0.03 and 0.002 mg-N/l, respectively) (Voytek et al., 1999).  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  concentrations are essentially constant with depth with exception of depths below 14m where  $\text{ClO}_4^-$  concentrations decrease (Figure 2.4.8). Chloride increases with depth until 12m below the surface beyond which it remains relatively constant.  $\text{ClO}_3^-/\text{Cl}^-$  and  $\text{ClO}_4^-/\text{Cl}^-$  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  $\text{SO}_4^{2-}$  below 14m strongly suggests that the sediments may support anoxic processes. This would be congruent with the reduction in  $\text{ClO}_4^-/\text{Cl}^-$  and  $\text{ClO}_3^-/\text{Cl}^-$  ratios.

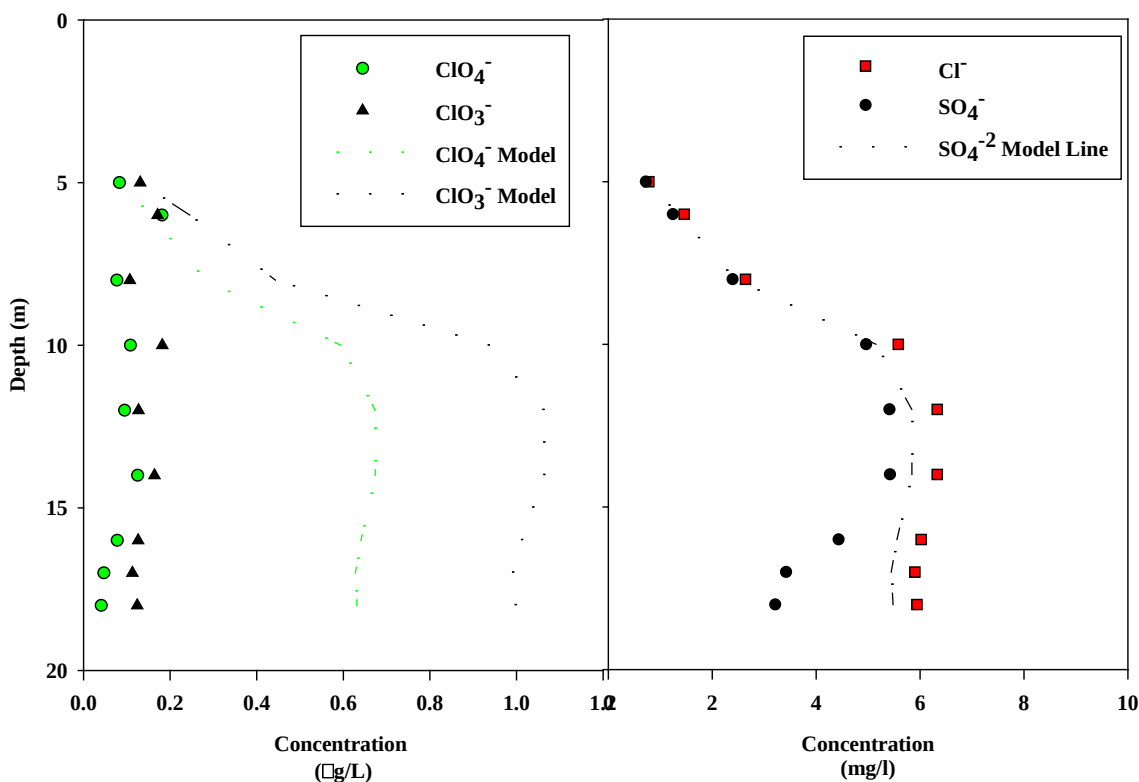


**Figure 2.4.8. Concentration profiles of  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  in Lake Miers, December 2008.** Dashed lines represent modeled concentrations based on maintaining surface water ratios of  $\text{ClO}_3^-$ ,  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  relative to  $\text{Cl}^-$ .

#### 2.4.6 Summary and Conclusions

$\text{ClO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  concentrations and ratios of  $\text{ClO}_3^-/\text{Cl}^-$  and  $\text{ClO}_4^-/\text{Cl}^-$  in surface waters that are similar to source streams, but suggest  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  reduction at depth or in the sediments. Lake Fryxell has  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  concentrations similar to input streams but  $\text{ClO}_3^-/\text{Cl}^-$  and  $\text{ClO}_4^-/\text{Cl}^-$  ratios much lower due to the large  $\text{Cl}^-$  source in bottom sediments due to its complete evaporation in the past. Based on the paucity of  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  in the deep waters, this lake appears to have supported  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  reduction at least back to the last draw down event.  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  concentrations in Lake Bonney are the highest of all the lakes reflecting the lake's greater age and concentration of  $\text{Cl}^-$ . Similar to  $\text{NO}_3^-$ ,  $\text{ClO}_4^-$  appears to be stable in the East Lobe and its concentration is highly correlated to  $\text{Cl}^-$  concentration. It is even possible that some  $\text{ClO}_4^-$  at depth is a remnant of the initial seawater that formed Lake Bonney. In the West Lobe  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  appear stable at depths above the chemocline but have or are experiencing reduction at the deepest depth similar to  $\text{NO}_3^-$ . Finally the concentrations of  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  are well correlated except in cases where reduction has occurred.

These lakes provide an excellent case study for  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  stable isotope evaluation could provide further clues as to the geochemical history of the lake water. Finally,  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in the Martian soil (Hecht et al., 2009).

## 2.5 Evaluate Cl and O Stable Isotope Signatures of UV and O<sub>3</sub> Generated Perchlorate

### 2.5.1 Background

As previously described, natural production of ClO<sub>4</sub><sup>-</sup> has been linked to atmospheric processes involving the oxidation of Cl and oxy-chlorine (ClO<sub>x</sub>) species by ultraviolet (UV) radiation and ozone (O<sub>3</sub>) (Kang et al., 2006, 2008, 2009; Rao et al., 2010, Wang et al., 2011). The pathways for ClO<sub>4</sub><sup>-</sup> formation are ambiguous and not well-defined, this more so for production of ClO<sub>4</sub><sup>-</sup> by O<sub>3</sub> oxidation than by photo-oxidation, of which there have been more proposed pathways. Although the actual ClO<sub>4</sub><sup>-</sup> 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<sub>4</sub><sup>-</sup> formation by both UV and O<sub>3</sub> as well as some of the suspected intermediates and other major products. Formation of the ClO<sub>4</sub><sup>-</sup> 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<sub>4</sub><sup>-</sup> has also been observed as a result of reactions involving chlorine monoxide (ClO) and chlorine dioxide (ClO<sub>2</sub>) radicals on the surface of sulfuric acid (Jaegl et al., 1996). The factors affecting ClO<sub>4</sub><sup>-</sup> formation can all contribute to fractionation effects that can lead to the variation observed in natural ClO<sub>4</sub><sup>-</sup> isotopic compositions.

At present there are no studies related to the potential for different ClO<sub>4</sub><sup>-</sup> formation pathways to produce distinct variations in ClO<sub>4</sub><sup>-</sup> isotopic composition. The purpose of this study was to evaluate the potential for different formation processes to impact the stable isotopic composition of ClO<sub>4</sub><sup>-</sup> produced from UV or O<sub>3</sub> oxidation of Cl and oxy-chlorine species. Isotopic compositions for water (H<sub>2</sub>O), O<sub>3</sub>, ClO<sub>4</sub><sup>-</sup>, starting materials, and major end products were determined. The isotopic data were compared to the known groups of natural ClO<sub>4</sub><sup>-</sup> isotopic compositions to determine if different ClO<sub>4</sub><sup>-</sup> formation processes contribute to the variation in natural ClO<sub>4</sub><sup>-</sup> isotopic compositions observed.

### 2.5.2 Methods

#### 2.5.2.1 Preparation of Reactant Species

Aqueous solutions of hypochlorite (OCl<sup>-</sup>), Cl<sup>-</sup>, and chlorite (ClO<sub>2</sub><sup>-</sup>) were prepared from calcium hypochlorite (Ca(OCl)<sub>2</sub>; available chlorine ~65%, Sigma-Aldrich), sodium chloride (NaCl; ACS reagent, 99 + %; Sigma-Aldrich), and sodium chlorite (NaClO<sub>2</sub>; technical grade, 80 %; Sigma-Aldrich) salts. All Ca(OCl)<sub>2</sub> solutions were filtered through a 0.2μm filter (PVDF Membrane, Millipore) before being used in the O<sub>3</sub> and photo-oxidation experiments. Concentrations for reactant species were based on ClO<sub>4</sub><sup>-</sup> yields from previous studies (Kang et al., 2008; Rao et al., 2010, 2012; Wang et al., 2011).

The aqueous chlorine dioxide ( $\text{ClO}_2$ ) 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  $\text{NaClO}_2$ , the two scrubber bottles (used to capture impurities) with flaked  $\text{NaClO}_2$  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  $\text{ClO}_2$  (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  $\text{ClO}_2$  occurred. The  $\text{ClO}_2$  was generated by bubbling a smooth current of purified compressed air through the system and by adding 2 mL of  $\text{H}_2\text{SO}_4$  in 5 mL increments through 5 minute intervals.

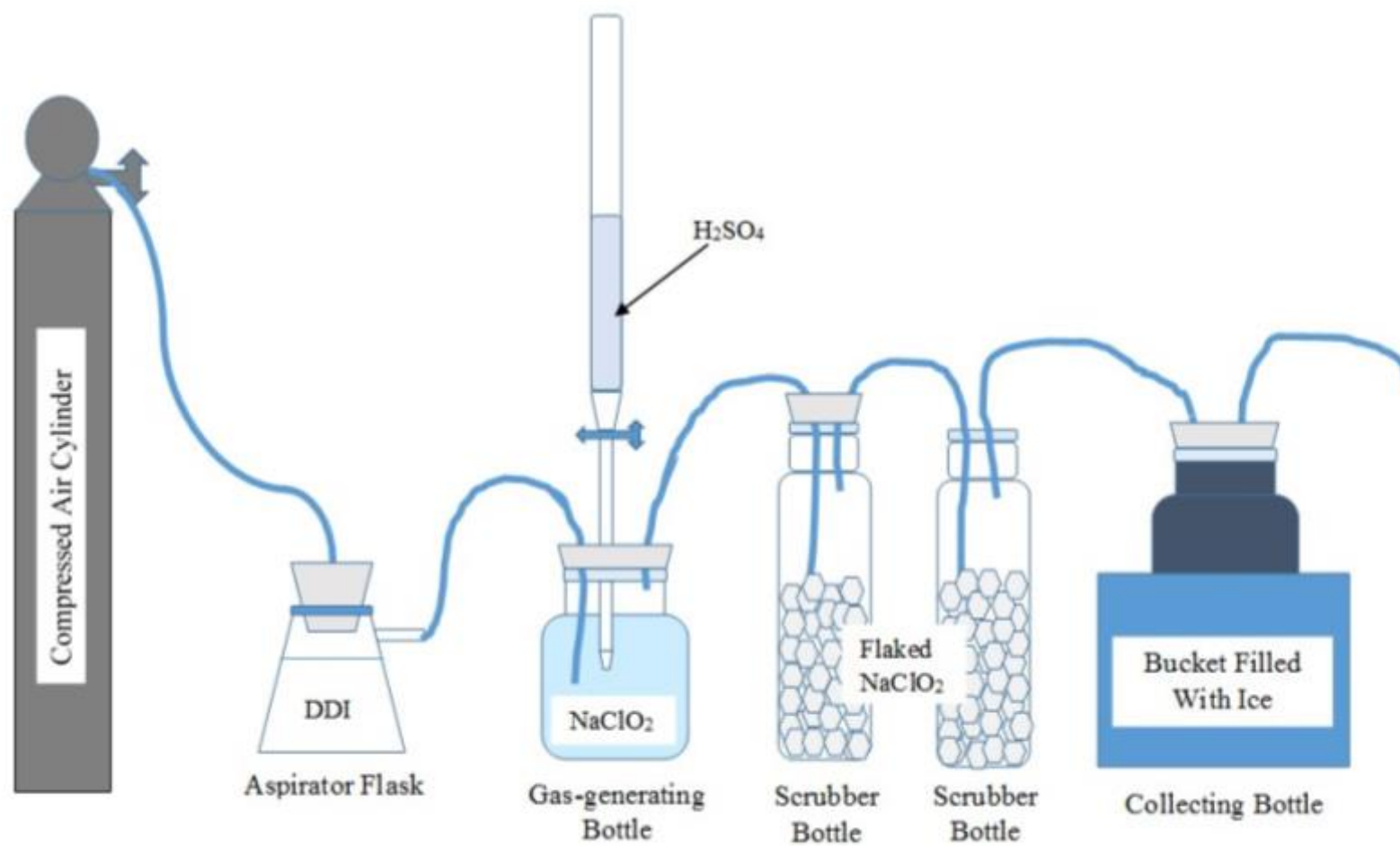


Figure 2.5.1 Chlorine dioxide gas-generating absorption system.

### 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 ( $\text{O}_2$ -purity > 99.999%) through an adjustable corona discharge  $\text{O}_3$  generator (Model OL80W, Ozone Services) and was bubbled through aqueous solutions of  $\text{ClO}_2$ ,  $\text{ClO}_2^-$ ,  $\text{OCl}^-$ , and  $\text{Cl}^-$  (Table 2.5.1 and Figure 2.5.2)

**Table 2.5.1. Experimental Conditions for  $\text{O}_3$  oxidation of various  $\text{Cl}/\text{ClO}_x$  species.**

| Replicate # | Reactant Species      | Species Mass (mg) | Volume (L) | Exposure Time |
|-------------|-----------------------|-------------------|------------|---------------|
| 1           | Dry $\text{Cl}^-$     | 514 041           | N/A        | ~ 1 month     |
| 1           | $\text{Cl}^-$ (aq)    | 211 200           | 32         | ~ 9 months    |
| 2           | $\text{Cl}^-$ (aq)    | 747 000           | 18         | ~ 9 months    |
| 1           | $\text{OCl}^-$ (aq)   | 13 000            | 1.8        | ~ 1 day       |
| 2           | $\text{OCl}^-$ (aq)   | 8 640             | 0.5        | ~ 1 day       |
| 3           | $\text{OCl}^-$ (aq)   | 11 782*           | 0.5        | ~ 1 day       |
| 1           | $\text{ClO}_2^-$ (aq) | 4 650             | 0.5        | ~ 2 – 20 hrs  |
| 2           | $\text{ClO}_2^-$ (aq) | 4 133             | 0.5        | ~ 2 – 20 hrs  |
| 1           | $\text{ClO}_2$ (aq)   | 38                | 0.5        | ~ 1.5 hrs     |
| 2           | $\text{ClO}_2$ (aq)   | 71                | 1          | ~ 1.5 hrs     |

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



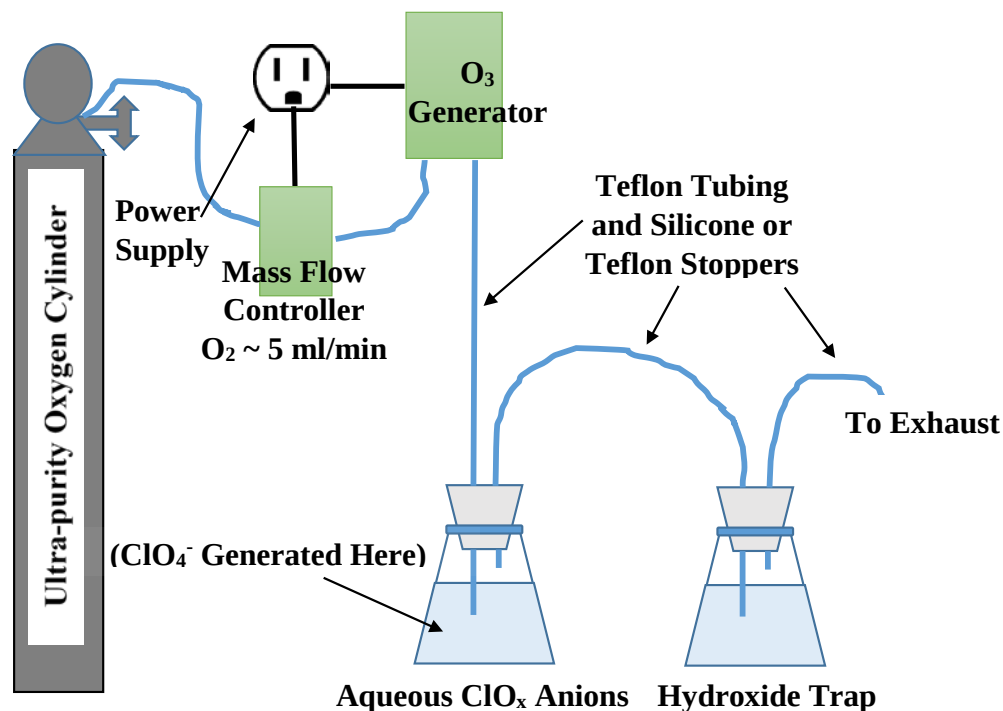


**Figure 2.5.2. Experimental set-up for aqueous  $O_3$  oxidation experiments.**

Generation of  $ClO_4^-$  by  $O_3$  oxidation of dry  $Cl^-$  ( $NaCl$ ; Certified ACS,  $\geq 99\%$ ; Fisher Chemical) was also performed. Ozone was passed through approximately 50 glass tubes containing  $\pm 10$  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  $ClO_4^-$ .

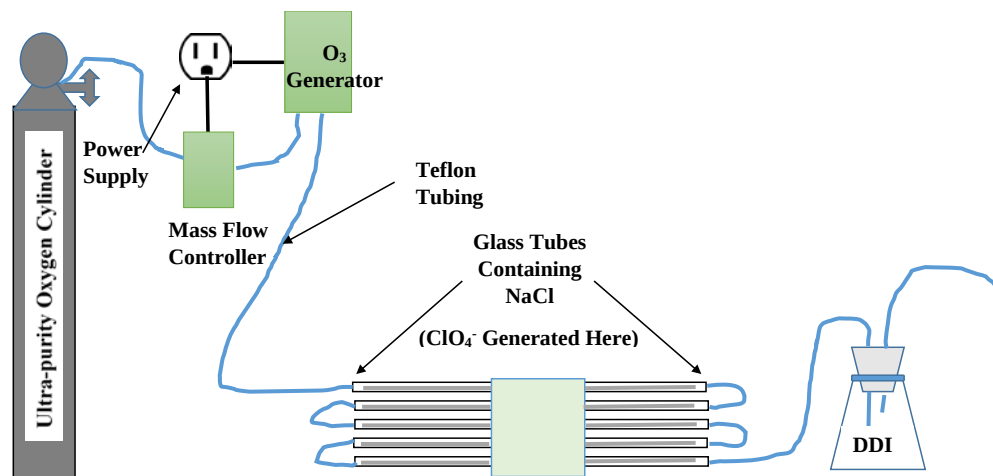


**Figure 2.5.3. Experimental set-up for O<sub>3</sub> oxidation of dry Cl<sup>-</sup>.**

### 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<sup>-</sup>, ClO<sub>2</sub><sup>-</sup>, and ClO<sub>2</sub> were irradiated with UV light ( $\lambda = 350$  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<sup>-</sup>, ClO<sub>2</sub><sup>-</sup>, and ClO<sub>2</sub>) was observed. All experiments were performed in duplicate and starting and ending solutions were analyzed for Cl<sup>-</sup>, OCl<sup>-</sup>, ClO<sub>2</sub><sup>-</sup>, ClO<sub>2</sub>, ClO<sub>3</sub><sup>-</sup>, and ClO<sub>4</sub><sup>-</sup>.

**Table 2.5.2. Experimental conditions for UV oxidation of various  $\text{ClO}_x$  species.**

| Replicate # | Reactant Species      | Species Mass (mg) | Volume (L) | Wavelength $\lambda$ | Exposure Time    |
|-------------|-----------------------|-------------------|------------|----------------------|------------------|
| 1           | $\text{OCl}^-$ (aq)   | 20 000            | 0.8        | 350 nm               | ~ 5 – 10 hrs     |
| 2           | $\text{OCl}^-$ (aq)   | 36 000            | 1          | 350 nm               | ~ 5 – 10 hrs     |
| 1           | $\text{ClO}_2^-$ (aq) | 8 000             | 0.3        | 350 nm               | ~ 1 – 3 hrs      |
| 2           | $\text{ClO}_2^-$ (aq) | 10 000            | 1          | 350 nm               | ~ 1 – 3 hrs      |
| 1           | $\text{ClO}_2$ (aq)   | 2 664             | 37         | 350 nm               | ~ 30 min – 3 hrs |
| 2           | $\text{ClO}_2$ (aq)   | 1 941             | 23.7       | 350 nm               | ~ 30 min – 3 hrs |

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

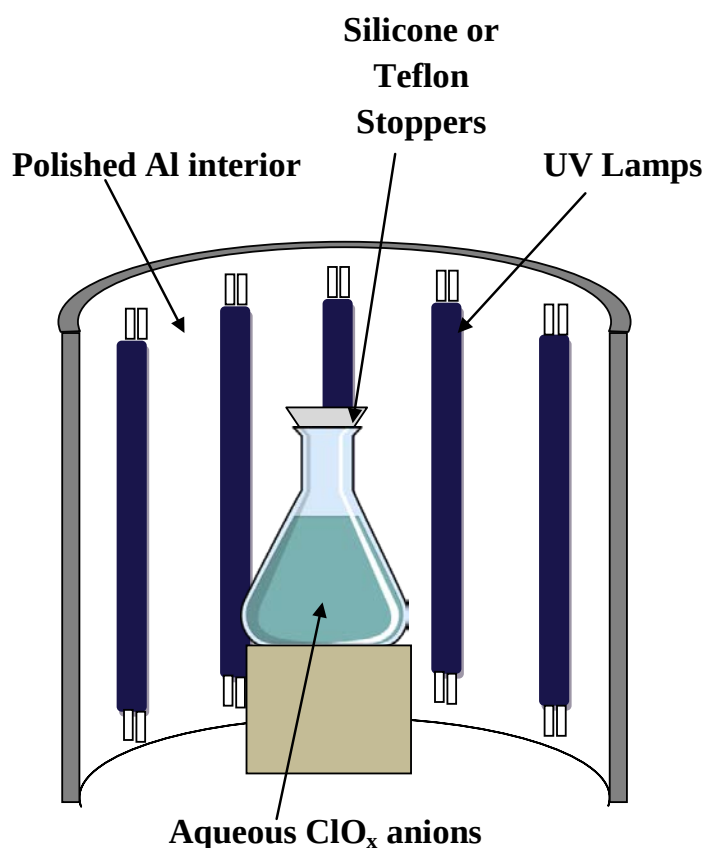


**Figure 2.5.4. Experimental set-up for UV oxidation experiments using the Rayonet photochemical chamber.**

#### 2.5.2.4 Sample Analysis

Perchlorate and  $\text{ClO}_3^-$  concentrations in starting and ending solutions were determined using sequential ion chromatography-mass spectroscopy/mass spectroscopy (IC-MS/MS) as described in Rao et al. (2007). Concentrations of  $\text{Cl}^-$  and  $\text{ClO}_2^-$  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 ( $\text{Na}_2\text{CO}_3$ )/1 mM sodium bicarbonate ( $\text{NaHCO}_3$ ) eluent while  $\text{ClO}_2^-$  was analyzed using a Dionex AS9-HC column and a 9 mM  $\text{Na}_2\text{CO}_3$  eluent. The limit of detection (LOD) for  $\text{Cl}^-$  and  $\text{ClO}_2^-$  was 0.5 mg/L.

Hypochlorite was measured as total  $\text{Cl}_2$  (total  $\text{Cl}_2 = \text{Cl}_2 + \text{HOCl} + \text{OCl}^-$ ) by buffering solution samples at pH 8.5 and spiking them with excess iodide ( $\text{I}^-$ ) to produce a tri-iodide species ( $\text{I}_3^-$ ) that could be measured using a spectrophotometer (DU® Series 700, Beckman Coulter®) at 351 nm ( $\epsilon = 2.54 \pm 0.02 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ ). The reaction stoichiometry between  $\text{Cl}_2$  and  $\text{I}_3^-$  was used to determine  $\text{OCl}^-$  concentration. The  $\text{ClO}_2$  in solutions was determined by both UV spectrometry at 359 nm ( $\epsilon = 1230 \pm 10 \text{ M}^{-1}\text{cm}^{-1}$ ) and iodometric titration with 0.025 N sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) (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  $\text{ClO}_4^-$  was collected (Gu et al., 2011). Ending solutions containing high amounts of  $\text{ClO}_3^-$  were first reduced so that their presence did not interfere with the uptake of  $\text{ClO}_4^-$  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 ( $\text{H}_2$ ) 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  $\text{ClO}_4^-$  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.  $\text{NO}_3^-$ , organics, carbonates) (Hatzinger et al., 2011). The adsorbed  $\text{ClO}_4^-$  was eluted from the preparative glass column with a combination of 1 M  $\text{FeCl}_3$  and 4 M HCl. The  $\text{FeCl}_3$ -HCl eluent solution (containing the eluted  $\text{ClO}_4^-$ ) contains high concentrations of organics, Fe, and other anions aside from  $\text{ClO}_4^-$ , 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  $\text{ClO}_4^-$  concentration in solution was in excess of 3 mg/mL. The  $\text{ClO}_4^-$  from the remaining purified solution was crystallized as cesium perchlorate ( $\text{CsClO}_4$ ) by adding  $\text{CsCl}$ . 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  $\text{ClO}_4^-$  salt ( $\text{CsClO}_4$ ) to CO that was measured in continuous-flow mode by isotope-ratio mass spectrometry (CO-CFIRMS) and on  $\text{O}_2$  produced by in vacuo decomposition of the  $\text{ClO}_4^-$  salt ( $\text{CsClO}_4$ ) 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  $\delta^{18}\text{O}$ ,  $\delta^{17}\text{O}$ , and  $\Delta^{17}\text{O}$  in terms of per mil (‰), defined as the deviation from the Vienna Standard Mean Ocean Water ( $\delta^{18}\text{O}_{\text{VSMOW}} = 0.0\text{‰}$ ) (Equations 1-3).

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

$$\delta^{17}\text{O} = \left[ \frac{\left[ \frac{n(^{17}\text{O})}{n(^{16}\text{O})} \right]_{\text{sample}}}{\left[ \frac{n(^{17}\text{O})}{n(^{16}\text{O})} \right]_{\text{VSMOW}}} - 1 \right] \times 1000 \quad (2)$$

$$\Delta^{17}\text{O} = k = \left( \left[ \frac{(1 + \delta^{17}\text{O})}{(1 + \delta^{18}\text{O})^\lambda} \right] - 1 \right) \times 1000 \quad (3)$$

Chlorine isotope ratios were analyzed at the Environmental Isotope Geochemistry Laboratory of the University of Illinois at Chicago by conversion of the  $\text{CsClO}_4$  salt into methyl chloride ( $\text{CH}_3\text{Cl}$ ) 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  $\delta^{37}\text{Cl}$  in terms of per mil (‰), defined as the deviation from that of Standard Mean Ocean Chloride (SMOC) (Equation 4).

$$\delta^{37}\text{Cl} = \left( \frac{\left[ \frac{n(^{37}\text{Cl})}{n(^{35}\text{Cl})} \right]_{\text{sample}}}{\left[ \frac{n(^{37}\text{Cl})}{n(^{35}\text{Cl})} \right]_{\text{SMOC}}} - 1 \right) \times 1000 \quad (4)$$

## 2.5.3 Results

### 2.5.3.1 Mass Balance and Product Yields

A mass balance on initial and final amounts of Cl of  $\text{ClO}_x$  species for UV and  $\text{O}_3$  mediated  $\text{ClO}_4^-$  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  $\text{OCl}^-$  was oxidized by  $\text{O}_3$ , 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.  $\text{ClO}_2$ ).

Production yields for  $\text{ClO}_x$  species detected in final solutions were also evaluated (Table 2.5.3).  $\text{ClO}_4^-$  production yields for the majority of experiments were within an order of magnitude of each other (0.01 – 0.15 %). The exceptions were the  $\text{O}_3$  aqueous and dry  $\text{Cl}^-$  experiments, which had the lowest  $\text{ClO}_4^-$  yields (note that not all  $\text{Cl}^-$  was reacted), and the  $\text{O}_3$ - $\text{ClO}_2$  (aq) and one UV- $\text{ClO}_2^-$  experiment(s), which had the highest  $\text{ClO}_4^-$  yields. It is unclear as to why one of the UV- $\text{ClO}_2^-$  replicates had a  $\text{ClO}_4^-$  yield 10-fold higher than the other, but it could be related to this treatment's higher initial  $\text{ClO}_2^-$  concentration as suggested by Kang et al. (2006) and Rao et al. (2012b). This UV- $\text{ClO}_2^-$  replicate also had a different product distribution than its replicate, suggesting that the difference in  $\text{ClO}_4^-$  yields may be due to different production mechanisms.

Oxidation of aqueous solutions of all starting  $\text{ClO}_x$  species produced  $\text{Cl}^-$  and  $\text{ClO}_3^-$  as major products with substantially smaller  $\text{ClO}_4^-$  yields.  $\text{Cl}^-$  was the dominant product for UV and  $\text{O}_3$  mediated oxidation of  $\text{OCl}^-$  and UV mediated oxidation of  $\text{ClO}_2^-$ .  $\text{ClO}_3^-$  was the major product for  $\text{O}_3$  mediated oxidation of  $\text{ClO}_2^-$  and UV and  $\text{O}_3$  mediated oxidation of  $\text{ClO}_2$  (aq). Except for the case of oxidation of solutions of  $\text{ClO}_2^-$  or  $\text{ClO}_2$ (aq) by  $\text{O}_3$  for which the  $\text{ClO}_3^-$  yield was > 80 %, yields of  $\text{Cl}^-$  and  $\text{ClO}_3^-$  generally were within a factor of 2–3 for  $\text{O}_3$  and UV mediated oxidation of aqueous solutions (Table 2.5.3). However, final products from the oxidation of  $\text{Cl}^-$  as a dry solid or in aqueous solution could not be evaluated as the majority of the  $\text{Cl}^-$  was unreacted at the end of the oxidation period. As such, a better representation of the product distribution for the  $\text{O}_3$ - $\text{Cl}^-$  (aqueous and dry) experiments would be the final molar  $\text{ClO}_4^-/\text{ClO}_3^-$  ratio, which for our study was estimated to be 0.0002 – 0.0003 and 5 for the aqueous and dry experiments, respectively. The  $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  was the only experimental condition for which  $\text{ClO}_4^-$  was produced in excess of  $\text{ClO}_3^-$ . Although the experimental conditions for our UV

and O<sub>3</sub> 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 ClO<sub>x</sub> species of initial and final solutions, Cl mass balance, and product yields for UV and O<sub>3</sub> experiments.**

| Ozone Experiments               |                      |          |                                    |                            |                                    |                                    |                          |                      |          |                                    |                                    |                                    |                                   |                |                         |             |                                       |                                       |                                       |
|---------------------------------|----------------------|----------|------------------------------------|----------------------------|------------------------------------|------------------------------------|--------------------------|----------------------|----------|------------------------------------|------------------------------------|------------------------------------|-----------------------------------|----------------|-------------------------|-------------|---------------------------------------|---------------------------------------|---------------------------------------|
| Initial Solution                |                      |          |                                    |                            |                                    |                                    |                          | Final Solution       |          |                                    |                                    |                                    | Mass Balance                      |                | Product Yields*         |             |                                       |                                       |                                       |
| Reactant Species                | Cl <sup>-</sup> mmol | OCI mmol | ClO <sub>2</sub> <sup>-</sup> mmol | ClO <sub>2</sub> (aq) mmol | ClO <sub>3</sub> <sup>-</sup> mmol | ClO <sub>4</sub> <sup>-</sup> mmol | in ClO <sub>x</sub> mmol | Cl <sup>-</sup> mmol | OCI mmol | ClO <sub>2</sub> <sup>-</sup> mmol | ClO <sub>3</sub> <sup>-</sup> mmol | ClO <sub>4</sub> <sup>-</sup> mmol | Total Cl in ClO <sub>x</sub> mmol | % Cl Accounted | Cl <sup>-</sup> Yield % | OCI Yield % | ClO <sub>2</sub> <sup>-</sup> Yield % | ClO <sub>3</sub> <sup>-</sup> Yield % | ClO <sub>4</sub> <sup>-</sup> Yield % |
| 1-OCI                           | 140.8                | 252.7    | < 0.55                             | NM                         | 11.2                               | 0.0005                             | 404.7                    | 300.4                | < 0.06   | < 0.55                             | 52.6                               | 0.09                               | 353.1                             | 87.2           | 63.17                   | -99.98      | 0.00                                  | 16.36                                 | 0.04                                  |
| 2-OCI                           | 37.2                 | 167.9    | < 0.55                             | NM                         | 1.5                                | 0.0001                             | 206.7                    | 54.4                 | 0.16     | < 0.55                             | 9.6                                | 0.02                               | 64.1                              | 31.0           | 10.20                   | -99.91      | 0.00                                  | 4.77                                  | 0.01                                  |
| 3-OCI                           | 41.2                 | 39.0     | < 0.55                             | NM                         | 1.7                                | 0.0002                             | 81.9                     | 81.3                 | 0.32     | < 0.55                             | 12.5                               | 0.03                               | 94.1                              | 114.9          | 102.68                  | -99.19      | 0.09                                  | 27.67                                 | 0.07                                  |
| 1-Cl <sup>-</sup>               | 5957.7               | < 0.06   | < 0.55                             | NM                         | < 0.60                             | 0.0004                             | 5957.7                   | 5867.4               | < 0.06   | < 0.55                             | 222.4                              | 0.05                               | 6089.9                            | 102.2          | -1.52                   | 0.00        | 0.00                                  | 3.72                                  | < 0.001                               |
| 1-Cl <sup>-</sup>               | 21071.9              | < 0.06   | < 0.55                             | NM                         | < 0.60                             | 0.0005                             | 21071.9                  | 21071.9              | < 0.06   | < 0.55                             | 94.9                               | 0.03                               | 21166.9                           | 100.5          | 0.00                    | 0.00        | 0.00                                  | 0.45                                  | < 0.001                               |
| 1-ClO <sub>2</sub> <sup>-</sup> | 18.2                 | NM       | 68.9                               | NM                         | 0.86                               | 0.0001                             | 88.0                     | 31.2                 | NM       | < 0.55                             | 65.9                               | 0.06                               | 97.2                              | 110.5          | 18.92                   | 0.00        | -99.63                                | 94.36                                 | 0.09                                  |
| 1-ClO <sub>2</sub> <sup>-</sup> | 19.2                 | < 0.06   | 61.3                               | NM                         | < 0.60                             | < 0.0001                           | 80.4                     | 27.7                 | < 0.06   | < 0.55                             | 60.5                               | 0.07                               | 88.3                              | 109.7          | 13.88                   | < 0.003     | -99.94                                | 98.07                                 | 0.11                                  |
| 1-Dry Cl <sup>-</sup>           | 14500.4              | NM       | NM                                 | NM                         | NM                                 | NM                                 | 14500.4                  | 12646.0              | NM       | NM                                 | < 0.60                             | 0.04                               | 12646.0                           | 87.2           | -12.79                  | 0.00        | 0.00                                  | < 0.0001                              | < 0.001                               |
| 1-ClO <sub>2</sub> (aq)         | NM                   | < 0.06   | < 0.55                             | 0.57                       | < 0.60                             | < 0.0001                           | 0.57                     | NM                   | NM       | NM                                 | < 0.60                             | 0.02                               | 0.02                              | 3.9            | 0.00                    | -1.03       | -0.52                                 | 83.25                                 | 3.89                                  |
| 2-ClO <sub>2</sub> (aq)         | < 0.03               | < 0.06   | < 0.55                             | 1.06                       | < 0.60                             | < 0.0001                           | 1.1                      | < 0.03               | < 0.06   | < 0.55                             | 0.85                               | 0.03                               | 0.88                              | 82.9           | 0.00                    | 1.47        | 0.00                                  | 80.02                                 | 2.74                                  |

| UV Experiments                  |                      |          |                                    |                            |                                    |                                    |                          |                      |          |                                    |                                    |                                    |                                   |                |                         |             |                                       |                                       |                                       |
|---------------------------------|----------------------|----------|------------------------------------|----------------------------|------------------------------------|------------------------------------|--------------------------|----------------------|----------|------------------------------------|------------------------------------|------------------------------------|-----------------------------------|----------------|-------------------------|-------------|---------------------------------------|---------------------------------------|---------------------------------------|
| Initial Solution                |                      |          |                                    |                            |                                    |                                    |                          | Final Solution       |          |                                    |                                    |                                    | Mass Balance                      |                | Product Yields*         |             |                                       |                                       |                                       |
| Reactant Species                | Cl <sup>-</sup> mmol | OCI mmol | ClO <sub>2</sub> <sup>-</sup> mmol | ClO <sub>2</sub> (aq) mmol | ClO <sub>3</sub> <sup>-</sup> mmol | ClO <sub>4</sub> <sup>-</sup> mmol | in ClO <sub>x</sub> mmol | Cl <sup>-</sup> mmol | OCI mmol | ClO <sub>2</sub> <sup>-</sup> mmol | ClO <sub>3</sub> <sup>-</sup> mmol | ClO <sub>4</sub> <sup>-</sup> mmol | Total Cl in ClO <sub>x</sub> mmol | % Cl Accounted | Cl <sup>-</sup> Yield % | OCI Yield % | ClO <sub>2</sub> <sup>-</sup> Yield % | ClO <sub>3</sub> <sup>-</sup> Yield % | ClO <sub>4</sub> <sup>-</sup> Yield % |
| 1-OCI                           | 216.64               | 388.73   | < 0.55                             | NM                         | 17.26                              | 0.00080                            | 622.6                    | 521.86               | < 0.06   | < 0.55                             | 117.44                             | 0.52                               | 639.82                            | 102.8          | 78.52                   | -99.99      | 0.00                                  | 25.77                                 | 0.13                                  |
| 2-OCI                           | 389.96               | 699.71   | < 0.55                             | NM                         | 31.06                              | 0.00145                            | 1120.7                   | 729.48               | < 0.06   | < 0.55                             | 177.59                             | 0.35                               | 907.42                            | 81.0           | 48.52                   | -100.00     | -0.05                                 | 20.94                                 | 0.05                                  |
| 1-ClO <sub>2</sub> <sup>-</sup> | 37.17                | < 0.06   | 118.61                             | NM                         | 0.68                               | 0.00322                            | 156.5                    | 119.83               | < 0.06   | < 0.55                             | 59.67                              | 0.18                               | 179.68                            | 114.8          | 69.69                   | 0.00        | -98.75                                | 49.74                                 | 0.15                                  |
| 2-ClO <sub>2</sub> <sup>-</sup> | 46.46                | < 0.06   | 148.26                             | NM                         | 0.85                               | 0.00402                            | 195.6                    | 109.31               | < 0.06   | < 0.55                             | 70.10                              | 2.45                               | 181.86                            | 93.0           | 42.39                   | 0.01        | -99.95                                | 46.71                                 | 1.65                                  |
| 1-ClO <sub>2</sub> (aq)         | 3.05                 | < 0.06   | 3.44                               | 39.50                      | < 0.60                             | 0.00179                            | 46.0                     | 14.92                | < 0.06   | < 0.55                             | 25.98                              | 0.02                               | 40.91                             | 89.0           | 30.06                   | 0.00        | -7.31                                 | 64.60                                 | 0.03                                  |
| 2-ClO <sub>2</sub> (aq)         | 0.67                 | < 0.06   | < 0.55                             | 28.77                      | < 0.60                             | 0.00260                            | 29.4                     | 10.80                | < 0.06   | < 0.55                             | 13.53                              | 0.02                               | 24.35                             | 82.7           | 35.23                   | -0.01       | -0.50                                 | 45.13                                 | 0.05                                  |

NM = Not measured

N/Y = No Yield

\* Product Yields were calculated using the following equation: : Product Yield = (Final ClO<sub>x</sub><sup>y</sup>-Initial ClO<sub>x</sub><sup>y</sup>)/Main Reactant ClO<sub>x</sub><sup>y</sup>



### 2.5.3.2 Ozone Experiment Isotopes

#### ***Dry Chloride***

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

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

#### ***Aqueous Chloride***

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

The  $\delta^{18}\text{O}$  values of final total O ( $\approx \text{ClO}_3^-$ ) were  $\sim 60 - 75\text{‰}$  lower than those of  $\text{ClO}_4^-$ . The  $\Delta^{17}\text{O}$  values of final total O ( $\approx \text{ClO}_3^-$ ) were greater than zero but much lower ( $\sim 25\text{‰}$  to  $28\text{‰}$ ) than  $\text{ClO}_4^-$   $\Delta^{17}\text{O}$  values.

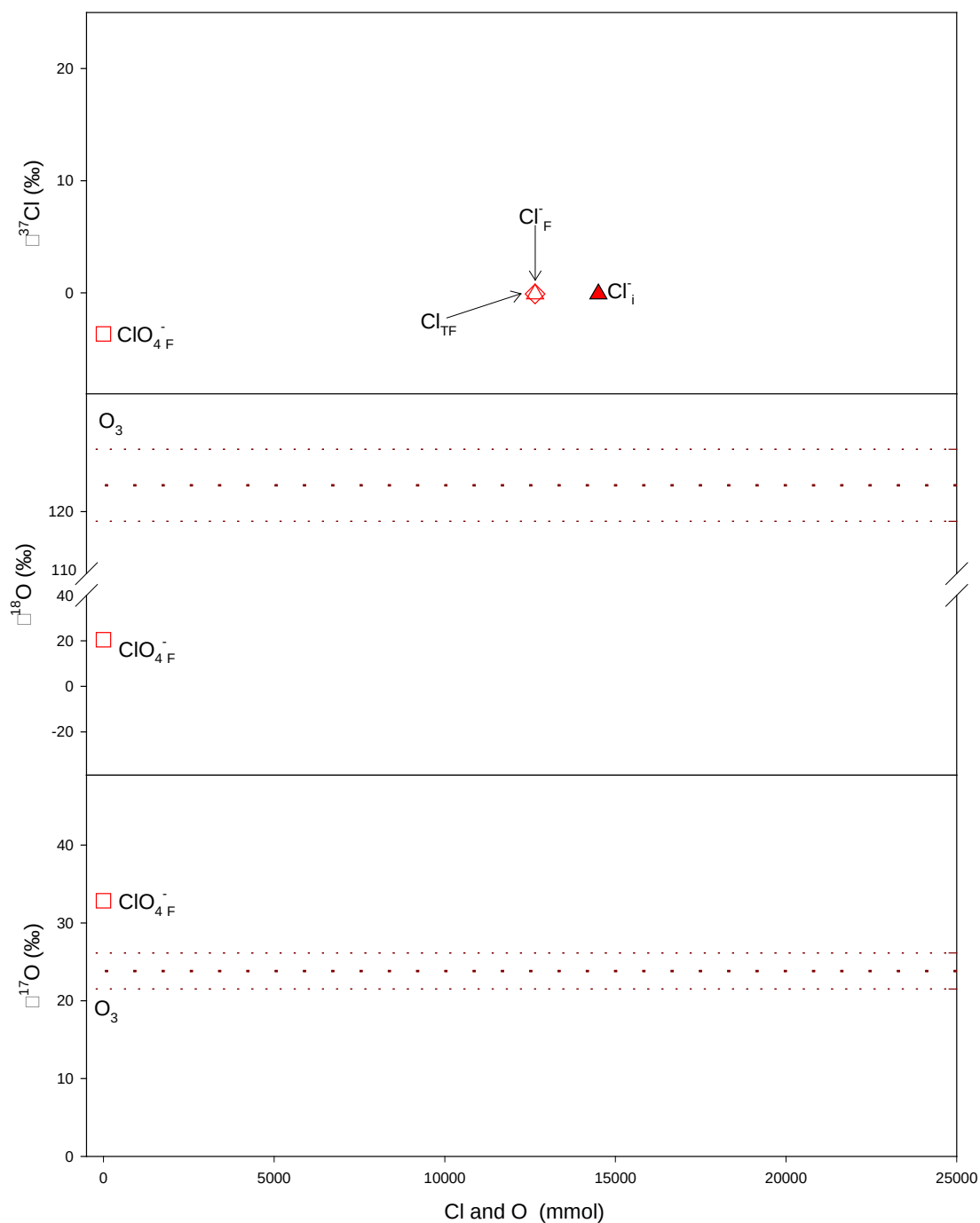


**Figure 2.5.5. Stable isotopic composition of initial and final species for  $\text{O}_3$  oxidized dry  $\text{Cl}^-$ .** Replicate experiments are represented by the different colors and different symbols represent the Cl and O of  $\text{ClO}_x$  species present in initial and final solutions, where  $\text{T}_\text{I}$  = total initial,  $\text{T}_\text{F}$  = total final,  $\text{I}$ =initial, and  $\text{F}$ =final. Note that the horizontal axis indicates the amount of Cl (for  $\delta^{37}\text{Cl}$ ) and the amount of O (for  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) in the initial and final experimental solutions.

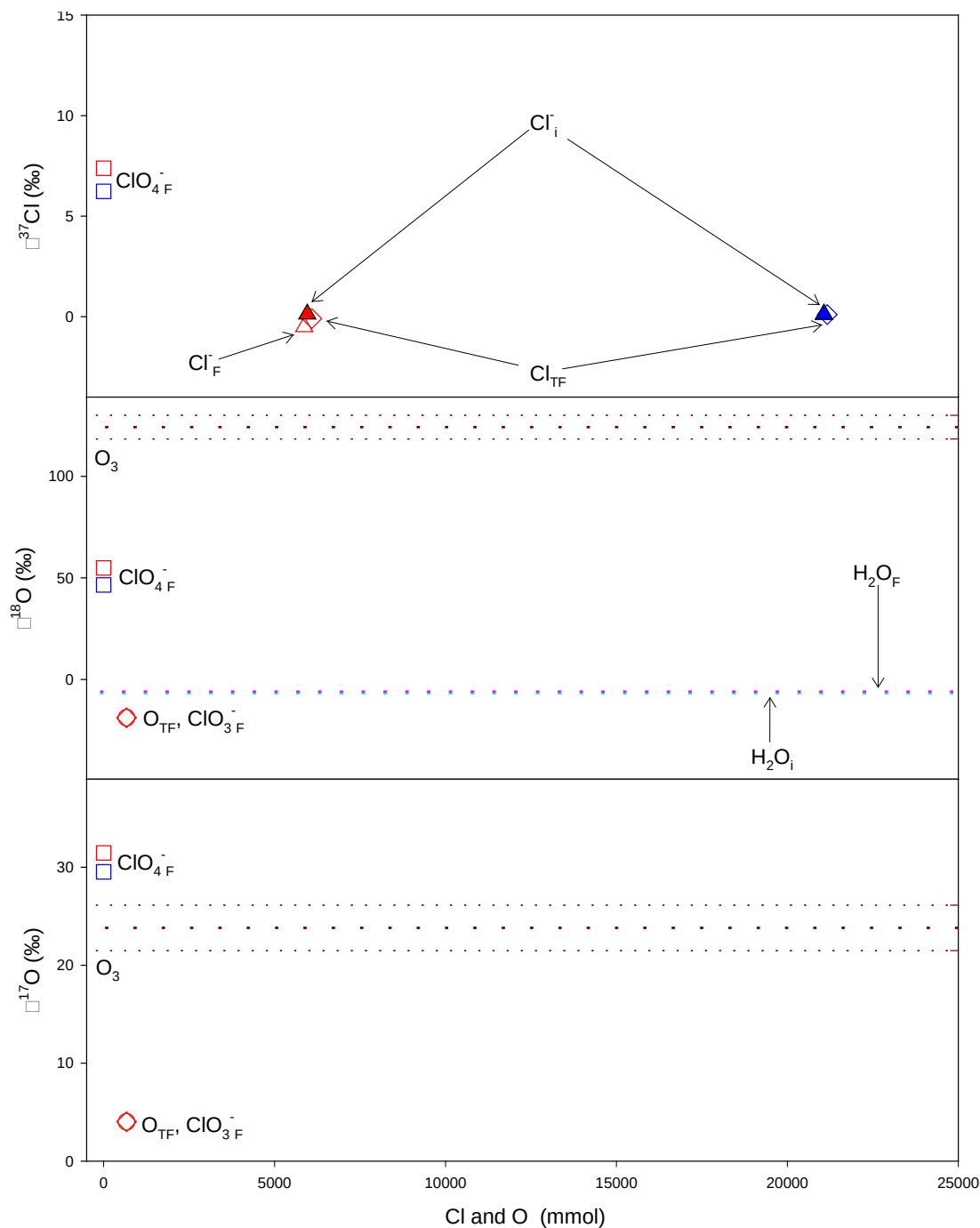


**Figure 2.5.6. Stable isotopic composition of initial and final species for  $\text{O}_3$  oxidized solutions of  $\text{Cl}^-$  (aq).** Replicate experiments are represented by the different colors and different symbols represent the Cl and O of  $\text{ClO}_x$  species present in initial and final solutions, where  $\text{T}_i$  = total initial,  $\text{T}_F$  = total final,  $\text{I}$ =initial, and  $\text{F}$ =final. Note that the horizontal axis indicates the amount of Cl (for  $\delta^{37}\text{Cl}$ ) and the amount of O (for  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) in the initial and final experimental solutions.

### ***Hypochlorite***

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

Final total O  $\delta^{18}\text{O}$  values were similar between all replicates as were final  $\text{ClO}_4^-$   $\delta^{18}\text{O}$  values, which were (32 ‰ – 47 ‰) higher than the final total O (essentially all  $\text{ClO}_3^-$ ). Initial total O  $\delta^{18}\text{O}$  values of two of the replicate experiments were similar and only slightly lower than final  $\text{ClO}_4^-$   $\delta^{18}\text{O}$  values. However, the third replicate experiment had a much lower initial total O  $\delta^{18}\text{O}$  value (~ 24 ‰), which is odd given the similar  $\delta^{18}\text{O}$  values for the final total O for all treatments and for final  $\text{ClO}_4^-$  for all treatments. This may suggest that the very low initial total  $\delta^{18}\text{O}$  values for the third replicate may be invalid.  $\Delta^{17}\text{O}$  values were similar for initial total O ( $\text{OCl}^- + \text{ClO}_3^-$ ) and final total O ( $\approx \text{ClO}_3^-$ ), with less than 1.5 ‰ variation. On the contrary,  $\text{ClO}_4^-$   $\Delta^{17}\text{O}$  values were much higher (15 ‰ – 20 ‰) compared to final  $\text{ClO}_3^-$  and initial and final total O.

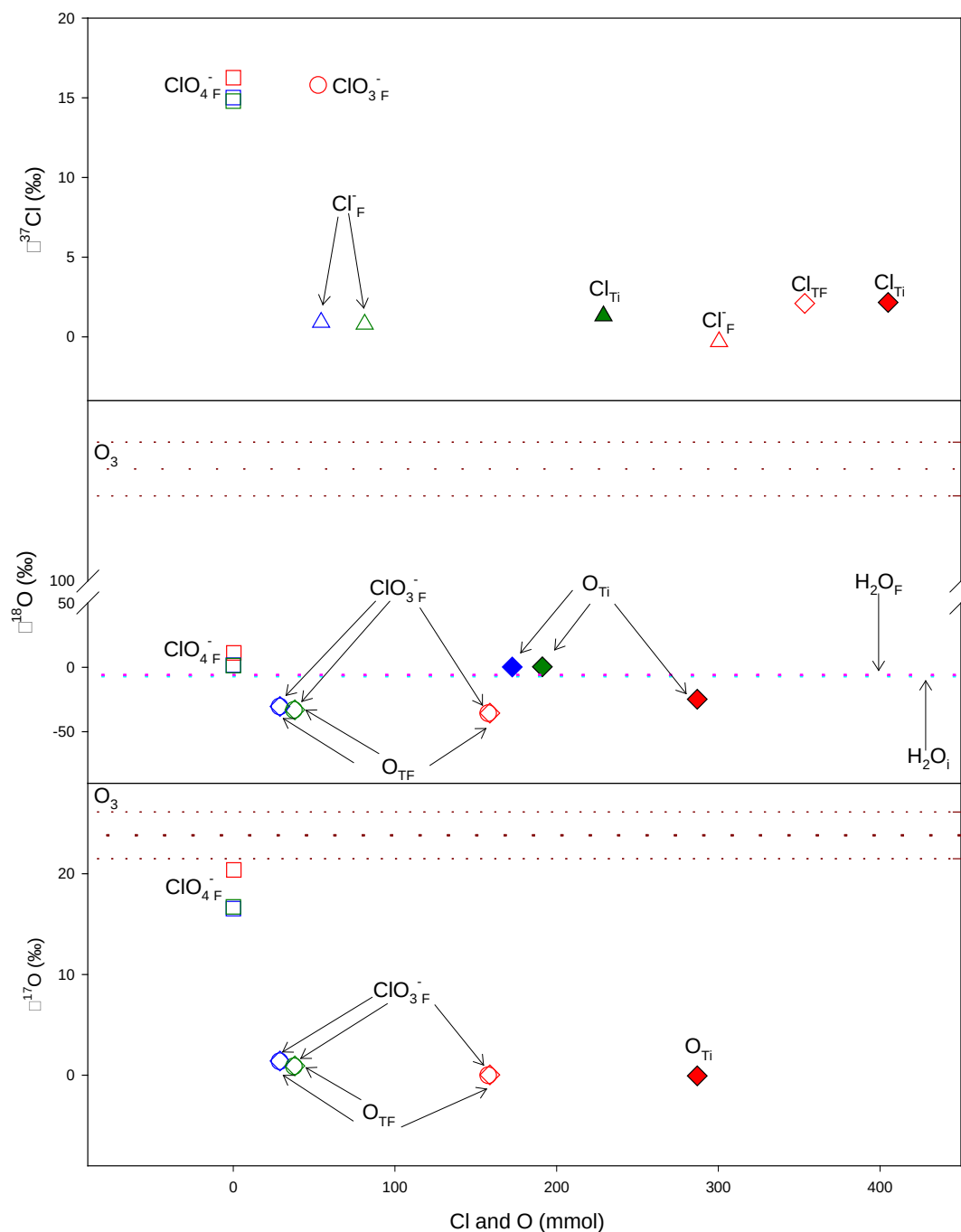


**Figure 2.5.7. Stable isotopic composition of initial and final species for  $\text{O}_3$  oxidized solutions of  $\text{OCl}^-$  (aq).** Replicate experiments are represented by the different colors and different symbols represent the Cl and O of  $\text{ClO}_x$  species present in initial and final solutions, where  $\text{T}_\text{I}$  = total initial,  $\text{T}_\text{F}$  = total final, I=initial, and F=final. Note that the horizontal axis indicates the amount of Cl (for  $\delta^{37}\text{Cl}$ ) and the amount of O (for  $\delta^{18}\text{O}$  and  $\delta^{17}\text{O}$ ) in the initial and final experimental solutions.

### ***Chlorite***

The  $\delta^{37}\text{Cl}$  values of initial total Cl ( $\text{ClO}_2^- + \text{Cl}^-$ ) and final total Cl ( $\text{Cl}^- + \text{ClO}_3^-$ ) were similar ( $< 0.5\text{‰}$ ) for all  $\text{ClO}_2^-$  replicates (Figure 2.5.8). Likewise,  $\delta^{37}\text{Cl}$  values of initial and final  $\text{Cl}^-$  were similar to one another ( $< 2\text{‰}$  difference), but were lower than the  $\delta^{37}\text{Cl}$  values of the initial total Cl ( $\text{ClO}_2^- + \text{Cl}^-$ ) by  $4\text{‰} - 6\text{‰}$ . Estimated (by mass balance)  $\delta^{37}\text{Cl}$  values for initial  $\text{ClO}_2^-$  and final  $\text{ClO}_3^-$  were similar to each other and higher than initial total Cl  $\delta^{37}\text{Cl}$  values by  $1.5\text{‰} - 2\text{‰}$ .  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  ( $+5\text{‰} - +6\text{‰}$ ) were higher than all other species.

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

### ***Chlorine Dioxide***

$\text{ClO}_2$  (aq) initial and final solution isotopes are not available, except for final  $\text{ClO}_4^-$  (Figure 2.5.9). The  $\delta^{37}\text{Cl}$  value of  $\text{ClO}_4^-$  ( $\sim +16\text{‰}$ ) was only determined for one experiment. The  $\delta^{18}\text{O}$  value of  $\text{ClO}_4^-$  varied between the experiments, with one of the replicates being  $4\text{‰}$  higher in  $\delta^{18}\text{O}$ .  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  were similar between both experiments at approximately  $+16.5\text{‰}$ .



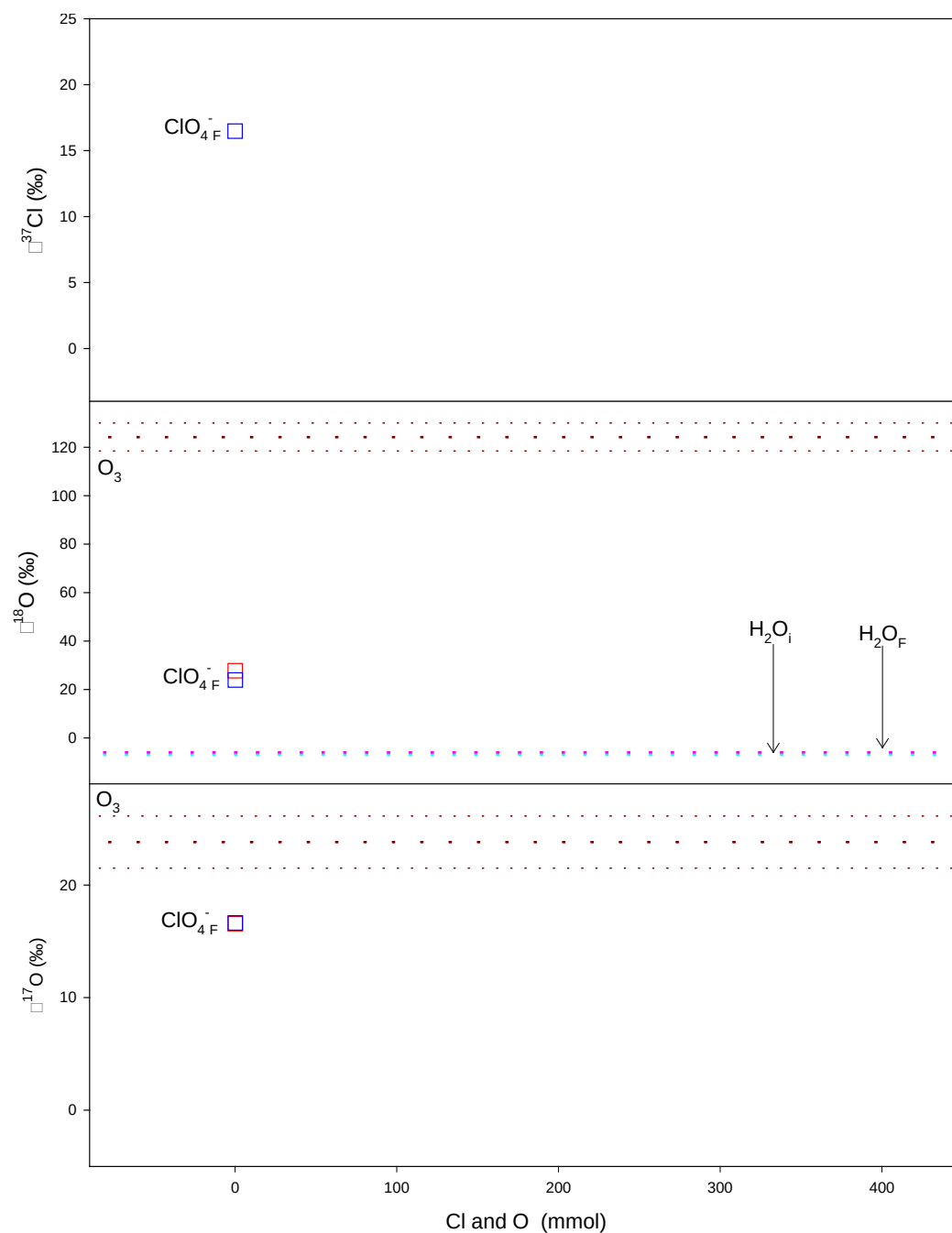


**Figure 2.5.9. Stable isotopic composition of initial and final species for  $\text{O}_3$  oxidized solutions of  $\text{ClO}_2$  (aq).** Replicate experiments are represented by the different colors and different symbols represent the Cl and O of  $\text{ClO}_x$  species present in initial and final 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  $\delta^{37}\text{Cl}$ ) and the amount of O (for  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) in the initial and final experimental solutions.



### 2.5.3.3 UV Experiment Isotopes

#### ***Hypochlorite***

Regardless of the starting  $\text{OCl}^-$  mass and production yields, the isotopic compositions ( $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ , and  $\Delta^{17}\text{O}$ ) of the reactants and products from photolysis ( $\lambda=350\text{ nm}$ ) of  $\text{OCl}^-$  solutions were essentially the same between both experiments (Figure 2.5.10). The  $\delta^{37}\text{Cl}$  of initial total Cl ( $\text{OCl}^- + \text{Cl}^- + \text{ClO}_3^-$ ), final total Cl ( $\text{Cl}^- + \text{ClO}_3^- + \text{ClO}_4^-$ ), initial  $\text{Cl}^-$ , and final  $\text{Cl}^-$  did not vary ( $< 1\text{ ‰}$ ).  $\delta^{37}\text{Cl}$  in  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  (based on mass balance) were higher ( $\sim 15\text{ ‰}$  and  $2\text{ ‰}$ , respectively) than initial and final total Cl or  $\text{Cl}^-$ .

The total O of  $\text{ClO}_x$  species in the final solution, which was almost solely composed of  $\text{ClO}_3^-$ , had  $\delta^{18}\text{O}$  values approximately  $11\text{ ‰} - 15\text{ ‰}$  lower than those of total O for the initial total  $\text{ClO}_x$  ( $\sim \text{OCl}^- + \text{Cl}^-$ ). Contrarily,  $\text{ClO}_4^-$   $\delta^{18}\text{O}$  was  $7\text{ ‰}$  higher than  $\delta^{18}\text{O}$  of initial total  $\text{ClO}_x$  ( $\sim \text{OCl}^- + \text{Cl}^-$ ).  $\Delta^{17}\text{O}$  values were essentially the same ( $-0.33\text{ ‰}$  to  $+0.45\text{ ‰}$ ) among all major species, both initial and final ( $\text{ClO}_4^-$ ,  $\text{ClO}_3^-$ , and total O in  $\text{ClO}_x$ ).

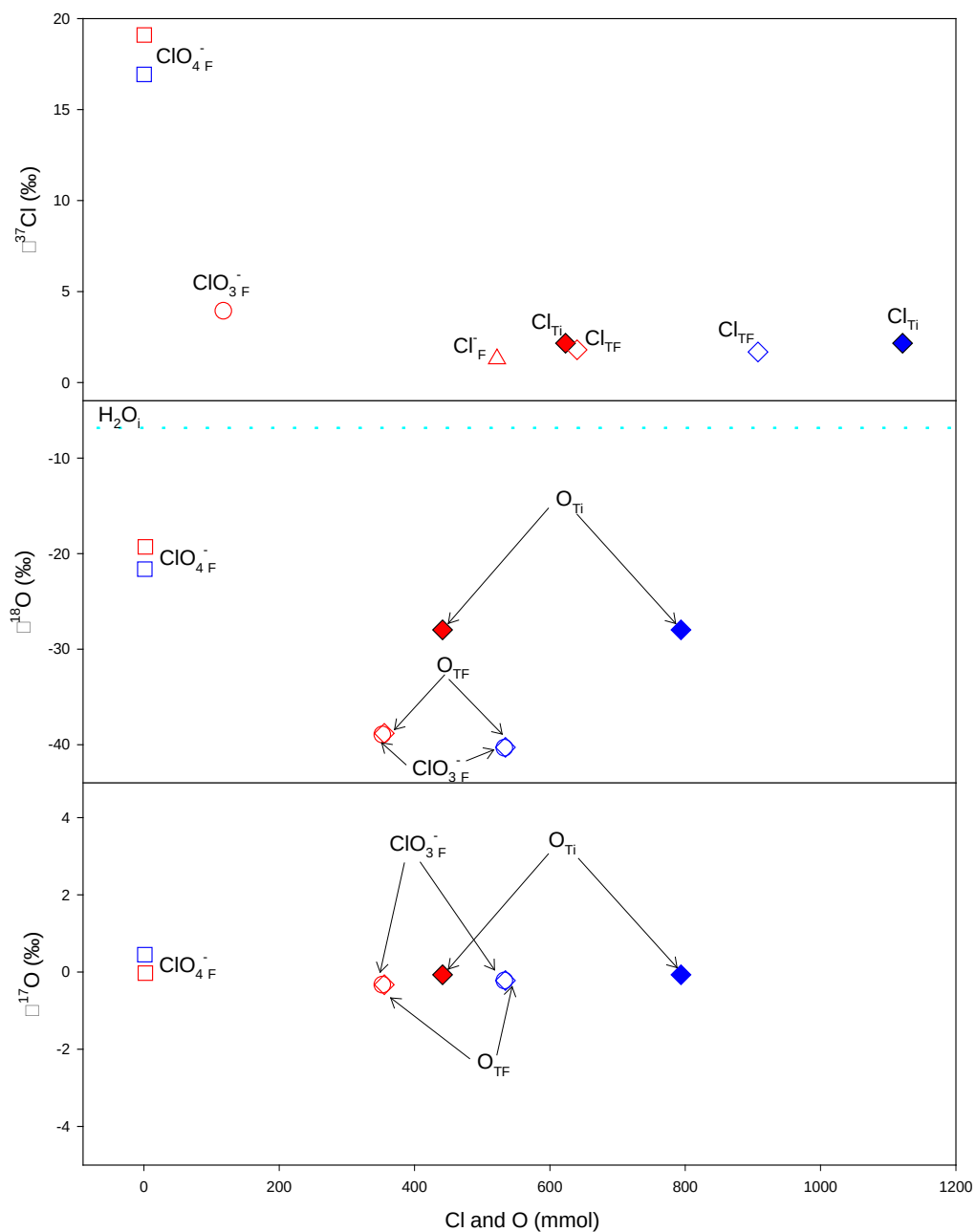


**Figure 2.5.10. Stable isotopic composition of initial and final species for solutions of  $\text{OCl}^-$  (aq) exposed to 350 nm irradiation.** Replicate experiments are represented by the different colors and different symbols represent the Cl and O of  $\text{ClO}_x$  species present in initial and final solutions, where  $\text{T}_\text{I}$  = total initial,  $\text{T}_\text{F}$  = total final,  $\text{I}$  = initial, and  $\text{F}$  = final. Note that the horizontal axis indicates the amount of Cl (for  $\delta^{37}\text{Cl}$ ) and the amount of O (for  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) in the initial and final experimental solutions.

### ***Chlorite***

There was little variation ( $< \pm 2$  ‰) in the  $\delta^{37}\text{Cl}$  values of initial total Cl ( $\text{ClO}_3^- + \text{ClO}_2^- + \text{Cl}^-$ ), final total Cl ( $\text{Cl}^- + \text{ClO}_3^- + \text{ClO}_4^-$ ), and final  $\text{Cl}^-$  for photolysis ( $\lambda = 350$  nm) of  $\text{ClO}_2^-$  solutions (Figure 2.5.11).  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  and estimated  $\text{ClO}_3^-$  (based on mass balance) were higher ( $\sim 24$  ‰ and 4 ‰, respectively) compared to final  $\text{Cl}^-$  and total Cl in initial and final solution.

There were small changes ( $< 3$  ‰) in  $\delta^{18}\text{O}$  values of initial total O ( $\sim \text{ClO}_2^-$ ) and final total O, which was primarily composed of  $\text{ClO}_3^-$ . Compared to initial total O ( $\sim \text{ClO}_2^-$ ), the  $\delta^{18}\text{O}$  value for  $\text{ClO}_4^-$  was higher ( $\sim 42$  ‰) and estimated  $\delta^{18}\text{O}$  value for  $\text{ClO}_3^-$  was lower ( $\sim 3$  ‰). No difference in  $\Delta^{17}\text{O}$  values between initial ( $\sim \text{ClO}_2^-$ ) and final total O ( $\sim \text{ClO}_3^-$ ) was observed, but the  $\Delta^{17}\text{O}$  value of  $\text{ClO}_4^-$  was smaller ( $< 2$  ‰).

### ***Chlorine Dioxide***

Isotopic compositions of initial  $\text{ClO}_2$  (aq) and final  $\text{ClO}_3^-$  (aq) were not able to be measured. The  $\delta^{37}\text{Cl}$  values ( $\sim +18$  ‰ and  $+20$  ‰),  $\delta^{18}\text{O}$  values ( $\sim +9$  ‰ and  $+13$  ‰), and  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  ( $\sim 0$  ‰) were similar between replicates (Figure 2.5.12).

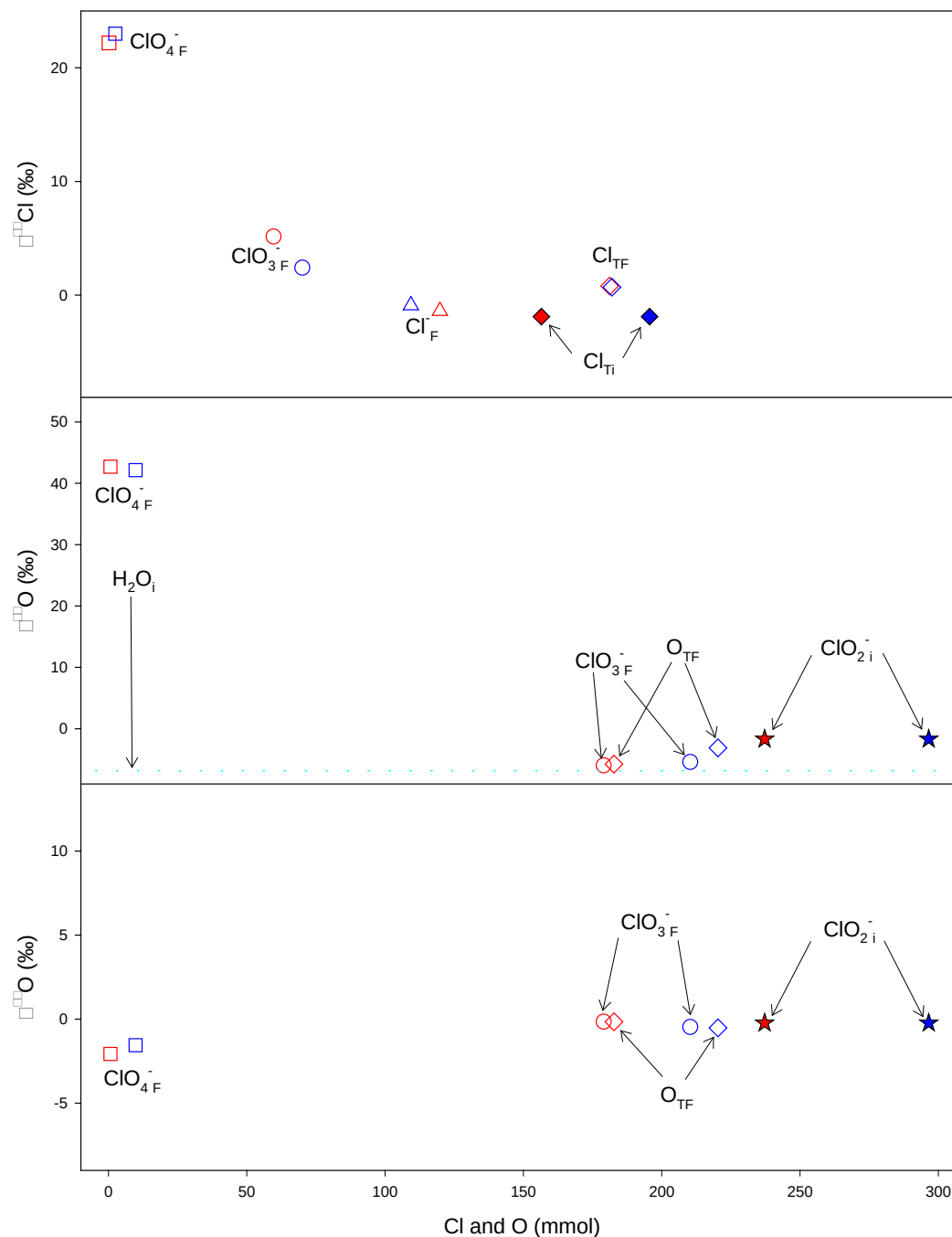


**Figure 2.5.11. Stable isotopic composition of initial and final species for solutions of  $\text{ClO}_2^-$  (aq) exposed to 350 nm irradiation.** Replicate experiments are represented by the different colors and different symbols represent the Cl and O of  $\text{ClO}_x$  species present in initial and final solutions, where  $\text{T}_i$  = total initial,  $\text{T}_f$  = total final,  $\text{I}$  = initial, and  $\text{F}$  = final. Note that the horizontal axis indicates the amount of Cl (for  $\delta^{37}\text{Cl}$ ) and the amount of O (for  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) in the initial and final experimental solutions.

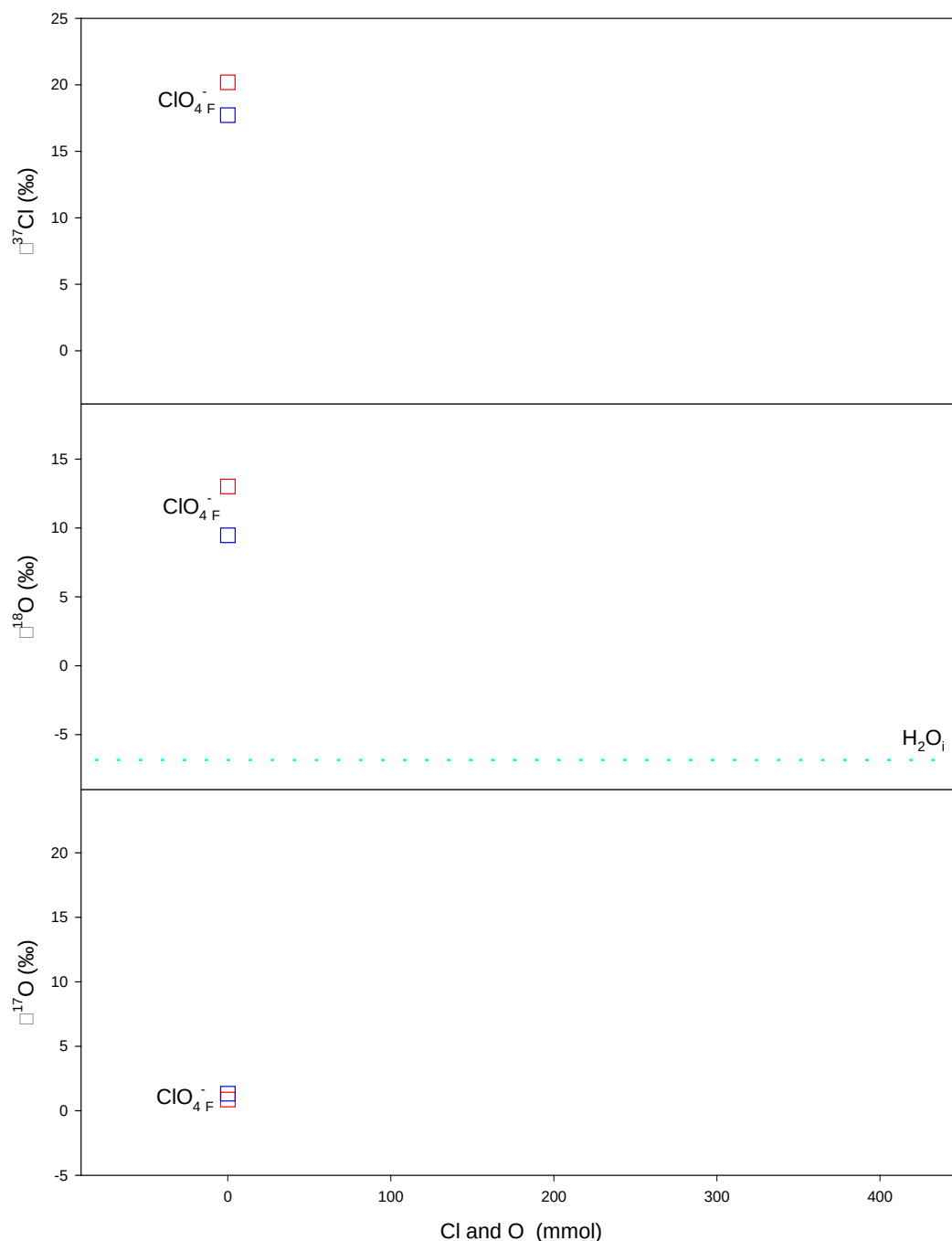


**Figure 2.5.12. Stable isotopic composition of initial and final species for solutions of  $\text{ClO}_2$  (aq) exposed to 350 nm irradiation.** Replicate experiments are represented by the different colors and different symbols represent the Cl and O of  $\text{ClO}_x$  species present in initial and 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  $\delta^{37}\text{Cl}$ ) and the amount of O (for  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) in the initial and final experimental solutions.

## 2.5.4 Discussion

### 2.5.4.1 Overall Observations

Perchlorate formed from UV and O<sub>3</sub> mediated oxidation processes generally had higher  $\delta^{37}\text{Cl}$  and  $\delta^{18}\text{O}$  values than all other ClO<sub>x</sub> 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 ClO<sub>x</sub> species were simply not measured. In only one of the experiments (dry Cl<sup>-</sup>), for which ClO<sub>4</sub><sup>-</sup> isotopes were measured, was the  $\delta^{37}\text{Cl}$  of the initial Cl<sup>-</sup> higher than in ClO<sub>4</sub><sup>-</sup>.

The same trend was observed for  $\delta^{18}\text{O}$  values of produced ClO<sub>4</sub><sup>-</sup> compared to those of the H<sub>2</sub>O used in the experiments. With the exception of the UV OCl<sup>-</sup> experiments, H<sub>2</sub>O typically bore a lower  $\delta^{18}\text{O}$  value than O in ClO<sub>4</sub><sup>-</sup>. The opposite was observed for the  $\delta^{18}\text{O}$  of O<sub>3</sub> generated in the lab versus  $\delta^{18}\text{O}$  of ClO<sub>4</sub><sup>-</sup> from O<sub>3</sub> experiments. The  $\delta^{18}\text{O}$  of O<sub>3</sub> (+ 124 ‰ ± 6 ‰) produced in the lab via the O<sub>3</sub> generator was always substantially higher than  $\delta^{18}\text{O}$  of produced ClO<sub>4</sub><sup>-</sup>, for which the highest  $\delta^{18}\text{O}$  value reported was ~ + 55 ‰.

The  $\Delta^{17}\text{O}$  of ClO<sub>4</sub><sup>-</sup> varied between the oxidation methods. In most cases, the  $\Delta^{17}\text{O}$  values of ClO<sub>4</sub><sup>-</sup> were below the  $\Delta^{17}\text{O}$  value of lab generated O<sub>3</sub> (+ 24.5 ‰). The exceptions were the dry and aqueous Cl<sup>-</sup> experiments for which values ranged from + 30 ‰ to + 33 ‰.

### 2.5.4.2 O<sub>3</sub> vs UV ClO<sub>4</sub><sup>-</sup> Isotopes

There was a discernible difference between the  $\Delta^{17}\text{O}$  of ClO<sub>4</sub><sup>-</sup> produced by O<sub>3</sub> mediated processes and the  $\Delta^{17}\text{O}$  of ClO<sub>4</sub><sup>-</sup> produced by UV mediated processes (Figures 2.5.13 and 2.5.14). While the  $\Delta^{17}\text{O}$  of UV ClO<sub>4</sub><sup>-</sup> was typically around zero (- 1.55 ‰ to + 1.33 ‰), the  $\Delta^{17}\text{O}$  of O<sub>3</sub> ClO<sub>4</sub><sup>-</sup> (+12 ‰ to + 33 ‰) was substantially higher, indicating the source of O for oxidation of Cl in ClO<sub>x</sub> precursor and intermediate species was different between UV and O<sub>3</sub> experiments.

Like  $\Delta^{17}\text{O}$  of ClO<sub>4</sub><sup>-</sup>, ClO<sub>4</sub><sup>-</sup> formed through O<sub>3</sub> mediated oxidation reactions tended to have higher  $\delta^{18}\text{O}$  values than ClO<sub>4</sub><sup>-</sup> formed through oxidation reactions produced by photolysis. The exception being the UV ClO<sub>2</sub><sup>-</sup> experiment, which had a ClO<sub>4</sub><sup>-</sup> with a higher  $\delta^{18}\text{O}$  value than the ClO<sub>4</sub><sup>-</sup> produced from O<sub>3</sub> oxidation of the same precursor species. In the case of  $\delta^{37}\text{Cl}$ , the  $\delta^{37}\text{Cl}$  of UV produced ClO<sub>4</sub><sup>-</sup> was always higher than the  $\delta^{37}\text{Cl}$  of ClO<sub>4</sub><sup>-</sup> produced from O<sub>3</sub> oxidation of the same precursor species.

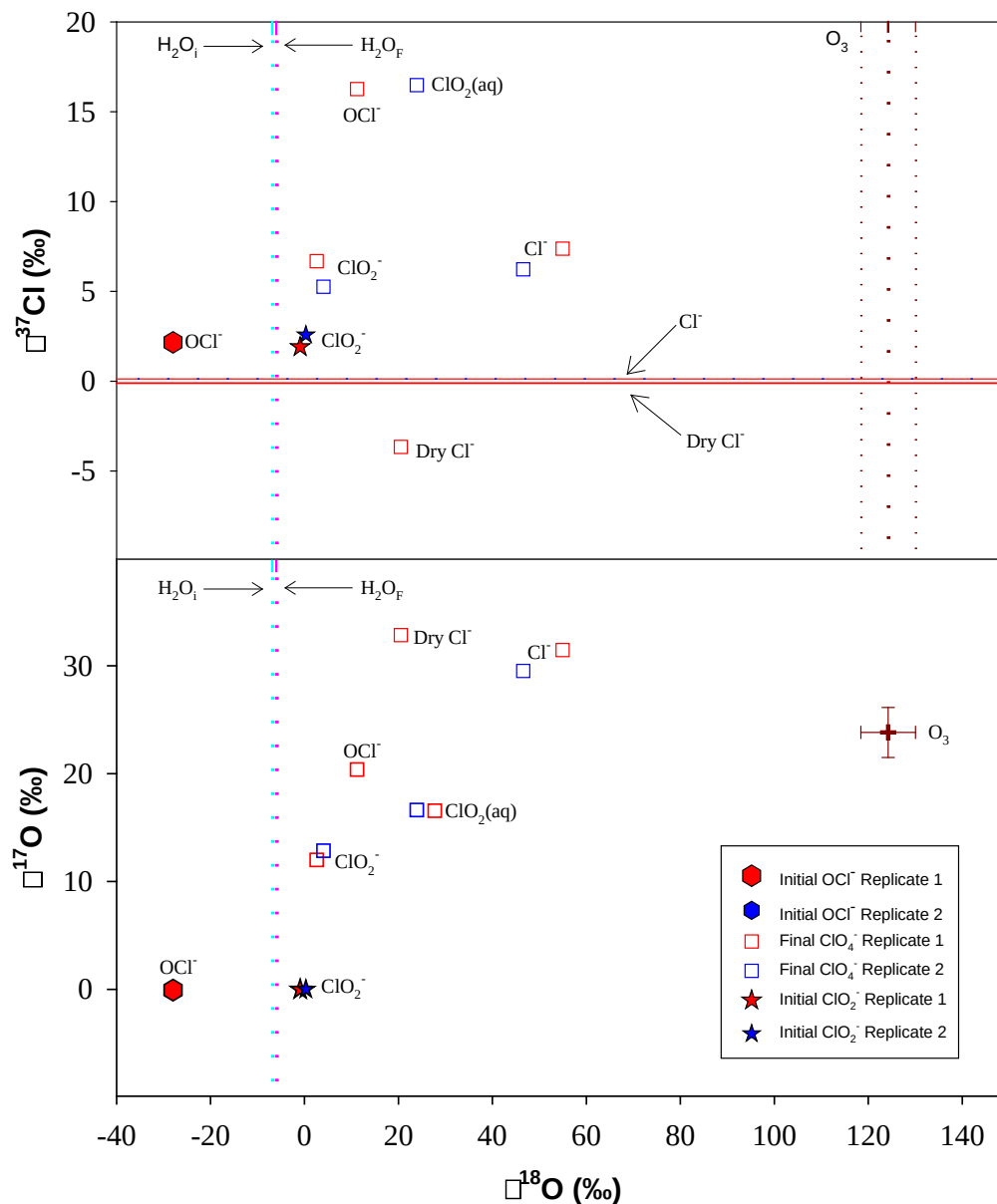


**Figure 2.5.13. Stable isotopic compositions ( $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ ,  $\Delta^{17}\text{O}$ ) of reactant species and final  $\text{ClO}_4^-$  for  $\text{O}_3$  oxidized solutions.** The vertical dashed light blue and pink lines represent the  $\delta^{18}\text{O}$  value for initial and final  $\text{H}_2\text{O}$ , respectively. The thick dark maroon dashed line represents the average  $\delta^{18}\text{O}$  value for  $\text{O}_3$  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  $\text{ClO}_4^-$ .

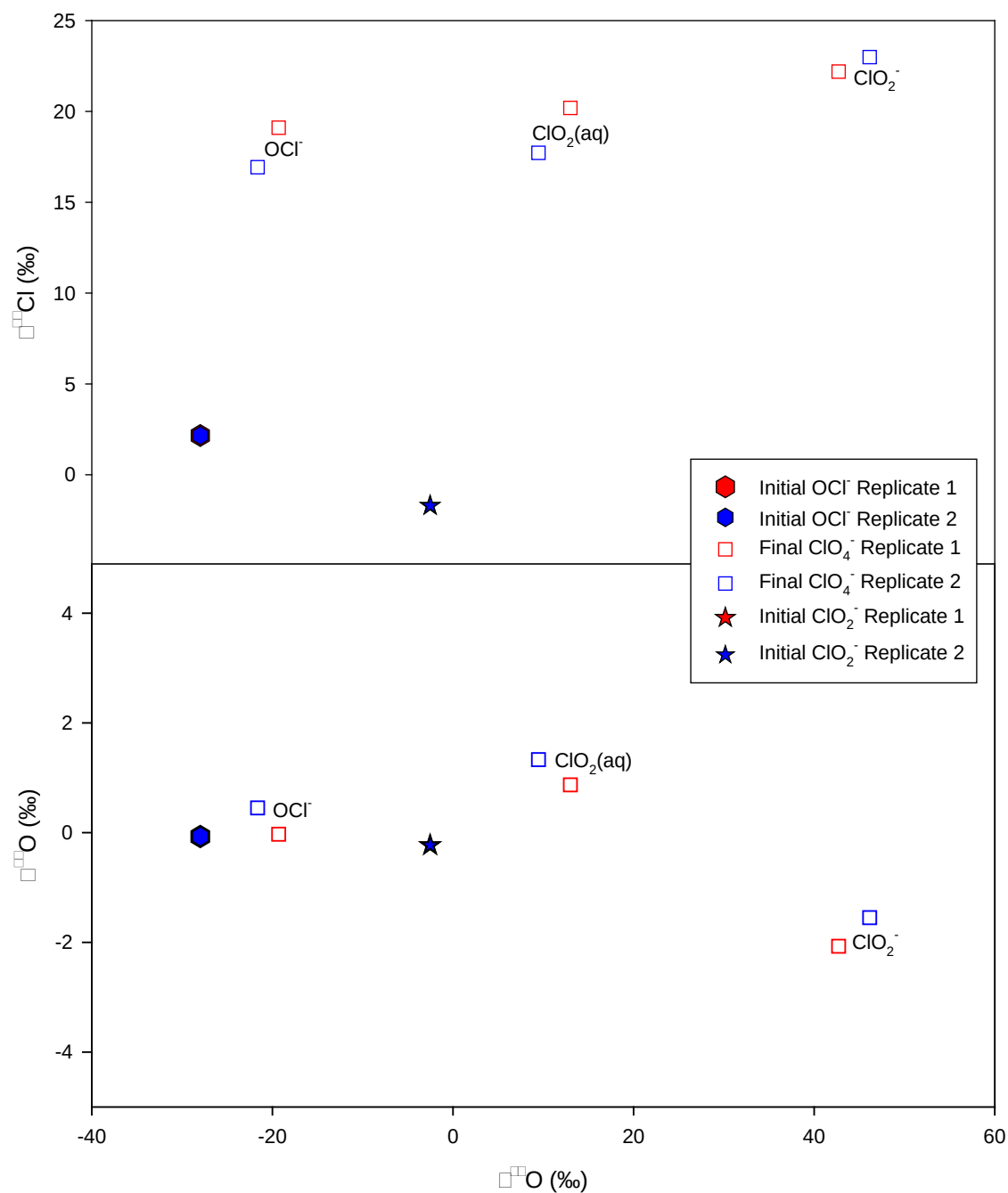


**Figure 2.5.14. Stable isotopic composition ( $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ ,  $\Delta^{17}\text{O}$ ) of reactant species and final  $\text{ClO}_4^-$  for UV oxidized solutions.** Replicate experiments are represented by the different colors and different symbols represent the reactant species and  $\text{ClO}_4^-$ .



#### 2.5.4.3 $\Delta^{17}\text{O}$ vs Precursor $\text{ClO}_x$ Species

There appears to be an inverse relationship between  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  formed by  $\text{O}_3$  mediated oxidation processes and the oxidation state of the Cl in the precursor  $\text{ClO}_x$  species (Figure 2.5.15). With the exception of the  $\text{O}_3$   $\text{ClO}_2^-$  experiments,  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  in  $\text{O}_3$  experiments decreased with increasing oxidation state of  $\text{ClO}_x$  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  $\text{Cl}^-$  (aqueous or dry), but there was a consistency with behavior of  $\Delta^{17}\text{O}$  values in  $\text{ClO}_4^-$  with the rest of the precursor species ( $\text{OCl}^-$ ,  $\text{ClO}_2^-$ , and  $\text{ClO}_2$ ) between both oxidation methods. In both UV and  $\text{O}_3$  (not considering  $\text{Cl}^-$ ),  $\text{ClO}_4^-$  formed from oxidation of  $\text{ClO}_2^-$  had the highest  $\Delta^{17}\text{O}$  values, followed by  $\text{ClO}_4^-$  formed from  $\text{OCl}^-$ , and lastly  $\text{ClO}_4^-$  formed from  $\text{ClO}_2$ . The  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  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 $\delta^{18}\text{O}$ vs Precursor $\text{ClO}_x$ Species

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

No connection was observed between oxidation state of the Cl in the precursor  $\text{ClO}_x$  species and  $\delta^{18}\text{O}$  values of  $\text{ClO}_4^-$  in the UV experiments. Nevertheless, it should be noted that  $\text{ClO}_4^-$  formed from UV oxidation of  $\text{ClO}_2^-$  had exceedingly high  $\delta^{18}\text{O}$  values, almost as high as those reported for  $\text{ClO}_4^-$  formed from  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq) (Figure 2.5.15b). On the contrary,  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  produced by UV oxidation of  $\text{ClO}_2^-$  were lower than any of the other UV experiments (Figure 2.5.12c).

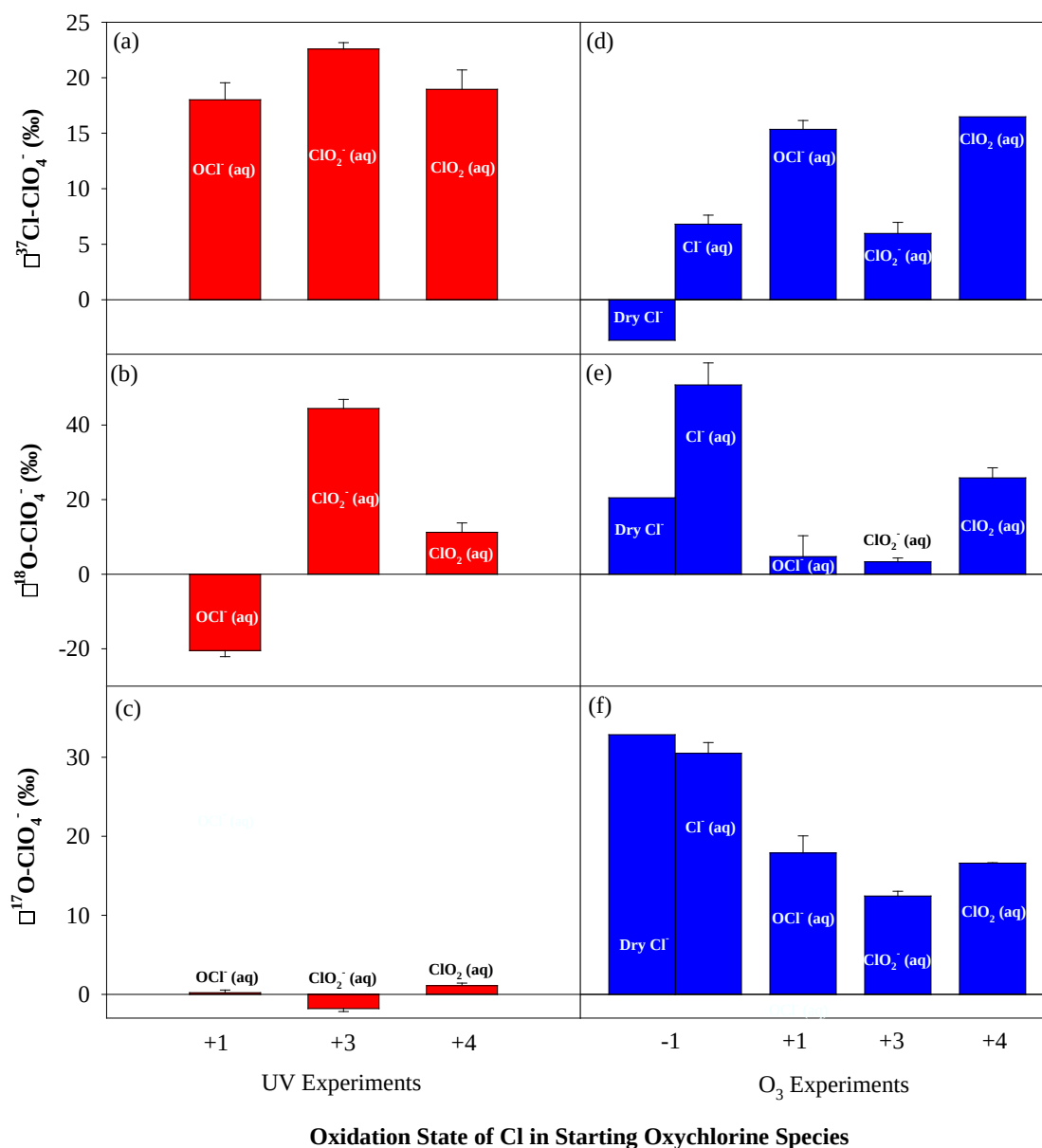


**Figure 2.5.15. Relationship between  $\delta^{37}\text{Cl}$  of final  $\text{ClO}_4^-$  produced by UV and  $\text{O}_3$  mediated oxidation of  $\text{Cl}^-$  (s),  $\text{Cl}^-$  (aq),  $\text{OCl}^-$  (aq),  $\text{ClO}_2^-$  (aq),  $\text{ClO}_2$  (aq) and the oxidation state of Cl in precursor  $\text{ClO}_x$  species.** UV experiment data (red bars) are presented in graphs a, b, and c while the  $\text{O}_3$  experiment data (blue bars) are presented in graphs d, e, and f. The name of the  $\text{ClO}_x$  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 $\text{ClO}_x$ Species

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

#### 2.5.4.6 Dry vs Aqueous Chloride

There was a clear difference between  $\text{ClO}_4^-$  formed from  $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  and  $\text{Cl}^-$  in aqueous solution (Figure 2.5.15). Perchlorate formed from  $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  was depleted in  $\delta^{37}\text{Cl}$  and  $\delta^{18}\text{O}$  compared to  $\text{ClO}_4^-$  formed from  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq), but was enriched in  $\Delta^{17}\text{O}$ . This is inconsistent with expected  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  from  $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  given the measured  $\delta^{18}\text{O}$  values of the  $\text{ClO}_4^-$ .  $\text{O}_3$  mediated oxidation reactions with dry  $\text{Cl}^-$  also yielded  $\text{ClO}_4^-$  containing the only negative  $\delta^{37}\text{Cl}$  values measured in all of our experiments (including both  $\text{O}_3$  and UV oxidation of  $\text{ClO}_x$  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 $\text{ClO}_3^-$ Isotopes

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

Calculated final product  $\text{ClO}_3^-/\text{ClO}_4^-$  ratios in the  $\text{O}_3$  experiments ranged from 0.18 – 4431, with the higher and lower end of this range corresponding to the  $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  and  $\text{Cl}^-$  (aq), respectively. Of the  $\text{O}_3$  experiments for which  $\text{ClO}_3^-$   $\Delta^{17}\text{O}$  values were estimated ( $\text{Cl}^-$  (aq),  $\text{OCl}^-$ , and  $\text{ClO}_2^-$ ), no clear trend was discernible between  $\text{ClO}_3^-/\text{ClO}_4^-$  final product ratios and the  $\Delta^{17}\text{O}$  values of  $\text{ClO}_3^-$ . A similarity was however, observed between  $\text{ClO}_4^-$   $\Delta^{17}\text{O}$  values and  $\text{ClO}_3^-$   $\Delta^{17}\text{O}$  values with respect to oxidation of Cl in precursor species and final  $\text{ClO}_3^-/\text{ClO}_4^-$  ratios. Both

final  $\text{ClO}_3^-/\text{ClO}_4^-$  ratios and  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  were highest for the  $\text{O}_3 \text{Cl}^-(\text{aq})$  experiments, followed by those of the  $\text{O}_3 \text{OCl}^-$  experiments, and lastly the  $\text{O}_3 \text{ClO}_2^-$  experiments. This further suggests a model in which pathways with common initial reaction stages for  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  production exist.

#### 2.5.4.8 Isotopic Characteristics of $\text{ClO}_4^-$ and their Connection to Formation Pathways

The isotopic composition of precursor compounds involved in  $\text{ClO}_4^-$  formation via UV and  $\text{O}_3$  processes and/or fractionation caused by isotope exchange reactions or kinetic processes could be used to explain our  $\text{ClO}_4^-$  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  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  formed from UV and  $\text{O}_3$  oxidation processes indicate that the heavier  $^{37}\text{Cl}$  isotope was preferentially accumulated in  $\text{ClO}_4^-$  compared to initial species and other final products, with the exception of two experiments ( $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  and  $\text{OCl}^-$ ). 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 ( $\text{ClO}_4^-$ ). The two exceptions ( $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  and  $\text{OCl}^-$ ) may be indicative of kinetic isotope effects that cause enrichments of the heavier isotopes in the residual reactants. The difference in isotope compositions of  $\text{ClO}_4^-$  produced by  $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  versus those of aqueous  $\text{Cl}^-$  suggests that reactions involving  $\text{ClO}_x$  oxidation of solid compounds may not fractionate isotopes in the same way as  $\text{ClO}_x(\text{aq})$  species. It is possible that one may be kinetically based whereas the other might be equilibrium based.  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  also suggest that  $\text{ClO}_4^-$  may be formed by more than one pathway and its isotopic composition may be the result of a mixture of the compositions of  $\text{ClO}_4^-$  formed by each distinct pathway.

#### 2.5.4.9 Implications of $\text{ClO}_4^- \Delta^{17}\text{O}$ values in $\text{O}_3$ Experiments

Conclusions regarding the mechanisms responsible for the  $\text{ClO}_4^-$  isotopic compositions observed in our study were made by considering proposed alternative  $\text{O}_3\text{-ClO}_x$  oxidation reactions and evaluating them with respect to which  $\text{ClO}_4^-$  formation pathways are plausible based on the  $\Delta^{17}\text{O}$  values of the  $\text{ClO}_4^-$  produced in our  $\text{O}_3$  experiments.

##### ***$\text{O}_3$ -Dry Chloride***

It is difficult to evaluate the formation of  $\text{ClO}_4^-$  from  $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  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  $\text{O}_2$  cylinders or bound water on the solids plays a role in reactions leading to its formation.

The O<sub>3</sub> dry Cl<sup>-</sup> experiment was the only experiment that resulted in a ClO<sub>3</sub><sup>-</sup>/ClO<sub>4</sub><sup>-</sup> product molar ratio (0.18) less than 1. The O<sub>3</sub> oxidation of dry Cl<sup>-</sup> produced ClO<sub>4</sub><sup>-</sup> with the highest Δ<sup>17</sup>O values (+ 33 ‰), but the values were only slightly higher than ClO<sub>4</sub><sup>-</sup> produced by O<sub>3</sub> oxidation of aqueous Cl<sup>-</sup> (aq) (+ 29 ‰ to + 32 ‰). This suggests that not only does liquid H<sub>2</sub>O not need to be present for the formation of ClO<sub>4</sub><sup>-</sup> to occur but that all four O in ClO<sub>4</sub><sup>-</sup> are sourced from O<sub>3</sub>, as O<sub>3</sub> produced from the O<sub>3</sub> generator had a bulk Δ<sup>17</sup>O value of + 24.5 ‰.

From the proposed O<sub>3</sub>-ClO<sub>x</sub> 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 ClO<sub>4</sub><sup>-</sup> with four O from O<sub>3</sub> in the absence of water (Table 2.5.4; Figure 2.5.16). Due to missing reaction rates, little can be inferred regarding the ClO<sub>4</sub><sup>-</sup> pathways proposed. Aside from ClO<sub>4</sub><sup>-</sup>, the only other detectible product was ClO<sub>3</sub><sup>-</sup> for which we were able to identify at least one pathway that formed ClO<sub>3</sub><sup>-</sup> without reactions involving H<sub>2</sub>O (Figure 2.5.17). This pathway has the same initial reaction sequences as formation of ClO<sub>4</sub><sup>-</sup> but a different final reaction (Table 2.5.4; Rxn 29). Due to the low mass of ClO<sub>3</sub><sup>-</sup> produced, no ClO<sub>3</sub><sup>-</sup> Δ<sup>17</sup>O values are available.

There has been much debate regarding the position (central vs terminal O atom) of the Δ<sup>17</sup>O anomaly in the O<sub>3</sub> 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 ClO<sub>4</sub><sup>-</sup> Δ<sup>17</sup>O values (+ 29 ‰ to + 32 ‰) being higher than those of the generated O<sub>3</sub> (+ 24.5 ‰) support the theory that terminal O atoms in O<sub>3</sub> are preferentially enriched in the Δ<sup>17</sup>O anomaly and that the terminal O atoms carrying the anomaly are preferentially incorporated into the ClO<sub>x</sub> compounds. If we assume that site preference is on the terminal O atom and that the Δ<sup>17</sup>O of the terminal O atom is transferred to ClO<sub>4</sub><sup>-</sup>, then we could theoretically calculate the Δ<sup>17</sup>O values of ClO<sub>4</sub><sup>-</sup> that we would expect if there was complete or partial preference of the heavy isotope on the terminal O atom of O<sub>3</sub> 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 ClO<sub>4</sub><sup>-</sup> with a maximum Δ<sup>17</sup>O value of + 37 ‰. With no terminal site preference, the maximum Δ<sup>17</sup>O of ClO<sub>4</sub><sup>-</sup> would be + 24.5 ‰. Our ClO<sub>4</sub><sup>-</sup> Δ<sup>17</sup>O values (+ 29 ‰ to + 32 ‰) were between these two values, indicating there is at least some preferential incorporation of terminal O atoms from O<sub>3</sub> into ClO<sub>4</sub><sup>-</sup>.

$$\Delta^{17}O \text{ of terminal } O_3 \text{ atoms} = \frac{3}{2} \times \Delta^{17}O \text{ of bulk } O_3 \quad (5)$$

However, it is not possible to completely constrain site preference because it is also possible that the observed  $\Delta^{17}\text{O}$  value of  $\text{ClO}_4^-$  from  $\text{O}_3$  oxidation of dry  $\text{Cl}^-$  is less than the value for complete preference due to the addition of a small amount of  $\text{ClO}_4^-$  formed from reaction pathways incorporating three or less O from  $\text{O}_3$  or partial O exchange during the reactions.

**Table 2.5.4. Proposed reactions possibly leading to the formation of  $\text{ClO}_4^-$  via  $\text{O}_3$  oxidation of  $\text{ClO}_x$  species.**

| Reaction # | Reaction                                                                     | Reaction Rate                                                                                                                                                                                            | Method                                                                                                                                                                                    | Comments  | Source                                                                       |
|------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------------------------------------------------------------------------|
| 1          | $\text{O}_3 + \text{Cl}^- \rightarrow \text{OCl}^-/\text{HOCl} + \text{O}_2$ |                                                                                                                                                                                                          | Based on $\text{O}_3(\text{aq})$ depletion                                                                                                                                                | pH > 3    | Hoigné et al. (1985)<br>Levanov et al. (2003)                                |
| 2          | $\text{O}_3 + \text{OCl}^- \rightarrow \text{O}_2 + \text{ClO}_2^-$          | $K = 30 \text{ M}^{-1}\text{s}^{-1}$ (1985; 1996)                                                                                                                                                        | Based on $\text{O}_3(\text{aq})$ depletion                                                                                                                                                |           | Hoigné et al. (1985)<br>Siddiqui (1996)                                      |
| 3          | $\text{O}_3 + \text{ClO}_2^- \leftrightarrow \text{O}_3^- + \text{ClO}_2$    | $^a K_F = 8.2(4) \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ (2002)<br>$^b K_R = 1.8 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ (1984)<br>$^c K_F = (4 \pm 1) \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ (1985) | $^a$ Determined under pseudo-first order conditions using stopped flow spectrometry<br>$^b$ Pulse Radiolysis<br>$^c$ Pulse Radiolysis and Stopped Flow Spectrometry                       |           | Nicoson et al. (2002)<br>Klaning et al. (1985)<br>Bühler et al. (1984)       |
| 4          | $\text{ClO}_2 + \text{O}_3 \rightarrow \text{ClO}_3 + \text{O}_2$            | $K = (1.05 \pm 0.10) \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ (1985)                                                                                                                                     | Stopped flow spectroscopy of $\text{ClO}_2(\text{aq})$ and $\text{O}(\text{aq})$                                                                                                          |           | Klaning et al. (1985)                                                        |
| 5          | $\text{ClO}_3 + \text{ClO}_3 \rightarrow \text{Cl}_2\text{O}_6$              | $K = (4.5 \pm 0.8) \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ (1997)<br>$K = 3.7 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ (2005, 1989)                                                                     | Calculated from Laser Photolysis of $\text{ClO}_3^-(\text{aq})$ (1997)<br>Assumed diffusion control by using the expression of Smoluchowski and the Stokes-Einstein Equation (2005, 1989) | Estimated | Zuo et al. (1997)<br>Quiroga and Perissinotti (2005)<br>Sander et al. (1989) |

|    |                                                                              |                                                                    |                                                                                    |                                  |                                                         |
|----|------------------------------------------------------------------------------|--------------------------------------------------------------------|------------------------------------------------------------------------------------|----------------------------------|---------------------------------------------------------|
| 6  | $O_2ClOClO_3 + O_3 \rightarrow Cl_2O_7 + O_2$                                |                                                                    |                                                                                    |                                  | Sander et al. (1989)<br>Wiberg et al. (2001)            |
| 7  | $Cl_2O_7 + H_2O \rightarrow 2ClO_4^- + 2H^+$                                 |                                                                    |                                                                                    |                                  | Wiberg et al. (2001)                                    |
| 8  | $OH + HOCl \rightarrow OCl + H_2O$                                           | $K = (1.4 \pm 0.1) \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ (1997) | Pulse Radiolysis of HOCl and H <sub>2</sub> SO <sub>4</sub>                        |                                  | Zuo et al. (1997)                                       |
| 9  | $ClO + O_3 \rightarrow ClOO + O_2$                                           |                                                                    |                                                                                    |                                  | Vaida and Simon (1995)                                  |
| 10 | $ClO + ClO \rightarrow Cl + ClOO$<br>OR<br>$ClO + ClO \rightarrow Cl + OClO$ |                                                                    |                                                                                    |                                  | Sander et al. (1989)                                    |
| 11 | $Cl + O_3 \rightarrow ClO + O_2$                                             |                                                                    | Flash Photolysis                                                                   | wavelength<br>> 300 nm           | Sander et al. (1989)                                    |
| 12 | $ClO_3 + HO \rightarrow HClO_4$                                              |                                                                    | Boron Doped Diamond Film<br>Electrodes, speculated to occur in the<br>stratosphere | Considered                       | Hubler et al. (2014)<br>Simonatis and Heicklen (1975)   |
| 13 | $OCl + HO \rightarrow HClO_2$                                                |                                                                    | Boron Doped Diamond Film<br>Electrodes                                             | Considered                       | Hubler et al. (2014)                                    |
| 14 | $O_2ClOClO_3 + H_2O \rightarrow ClO_3^- + ClO_4^- + 2H^+$                    | $K = 180 \text{ M}^{-1}\text{s}^{-1}$                              | Assumed attack of water on the more<br>acidic chlorine                             | Adopted from<br>previous studies | Quiroga and Perissinotti (2005)<br>Wiberg et al. (2001) |



|    |                                                                                                                   |                                                                      |                                                                                                            |                               |                                                         |
|----|-------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------|---------------------------------------------------------|
| 15 | $OCIO + ClO \rightarrow ClOClO_2$                                                                                 | $K = 7.4 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$                    | Assumed diffusion control by using the expression of Smoluchoswski (Cite) and the Stokes-Einstein Equation | Estimated                     | Quiroga and Perissinotti (2005)<br>Sander et al. (1989) |
| 16 | $Cl_2O_3 + O_3 \rightarrow Cl_2O_4$                                                                               |                                                                      |                                                                                                            | Hypothesised<br>Considered    |                                                         |
| 17 | $Cl_2O_4 + H_2O \rightarrow HClO + ClO_4^- + H^+$                                                                 | $K = 180 \text{ M}^{-1}\text{s}^{-1}$                                | Estimated                                                                                                  |                               | Quiroga and Perissinotti (2005)<br>Wiberg et al. (2001) |
| 18 | $OCIO + Cl \rightarrow ClClO_2$                                                                                   | $K = 7.8 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$                    | Assumed diffusion control by using the expression of Smoluchoswski and the Stokes-Einstein Equation        | Estimated                     | Quiroga and Perissinotti (2005)                         |
| 19 | $Cl_2O_2 + O_3 \rightarrow Cl_2O_3$                                                                               |                                                                      |                                                                                                            | Hypothesised<br>Considered    |                                                         |
| 20 | $OCIO + ClO_3 \rightarrow O_2ClOClO_2$                                                                            | $K = 7.5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$                    | Assumed diffusion control by using the expression of Smoluchoswski and the Stokes-Einstein Equation        | Estimated                     | Quiroga and Perissinotti (2005)                         |
| 21 | $O_2ClOClO_2 + H_2O \rightarrow 2ClO_3^- + 2H^+$<br>OR<br>$O_3ClOClO + H_2O \rightarrow ClO_3^- + ClO_4^- + 2H^+$ | $K_{ClO_3^-} = 180 \text{ M}^{-1}\text{s}^{-1}$<br>$K_{ClO_4^-} = ?$ | Assumed attack of water on the more acidic chlorine                                                        | Adopted from previous studies | Quiroga and Perissinotti (2005)                         |
| 22 | $ClO + ClO_3 \rightarrow ClOClO_3$                                                                                | $K = 7.5 \times 10^9$                                                | Assumed diffusion control by using the expression of Smoluchoswski and the Stokes-Einstein Equation        | Estimated<br>Proposed         | Quiroga and Perissinotti (2005)<br>Sander et al. (1989) |
| 23 | $ClO + ClO + M \rightarrow ClOOC l + M$                                                                           |                                                                      |                                                                                                            |                               | von Hobe et al. (2009)<br>Sander et al. (1989)          |

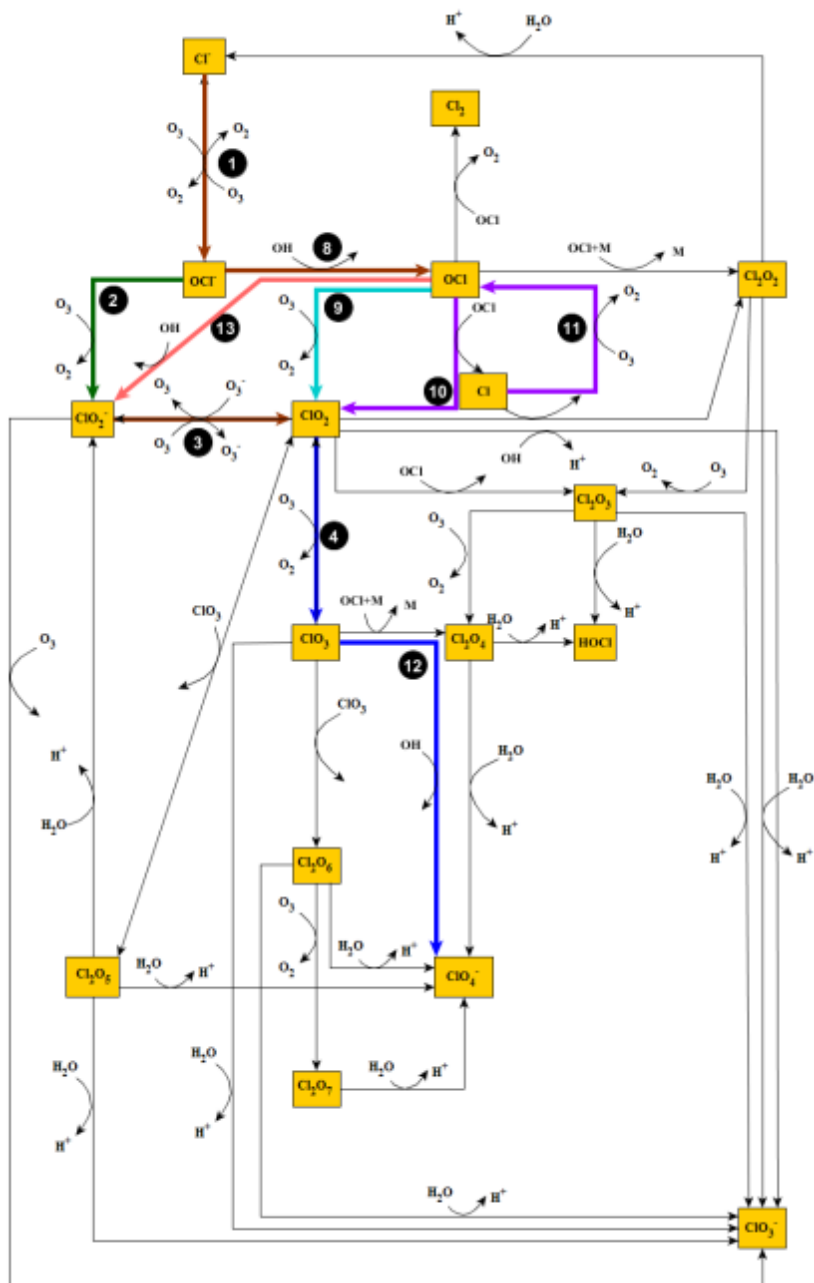


**Figure 2.5.16. Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (dry and aq).** Note the thick forest dark red lines indicate the initial reactions that some of the species may share while the dark blue lines indicate the 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  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

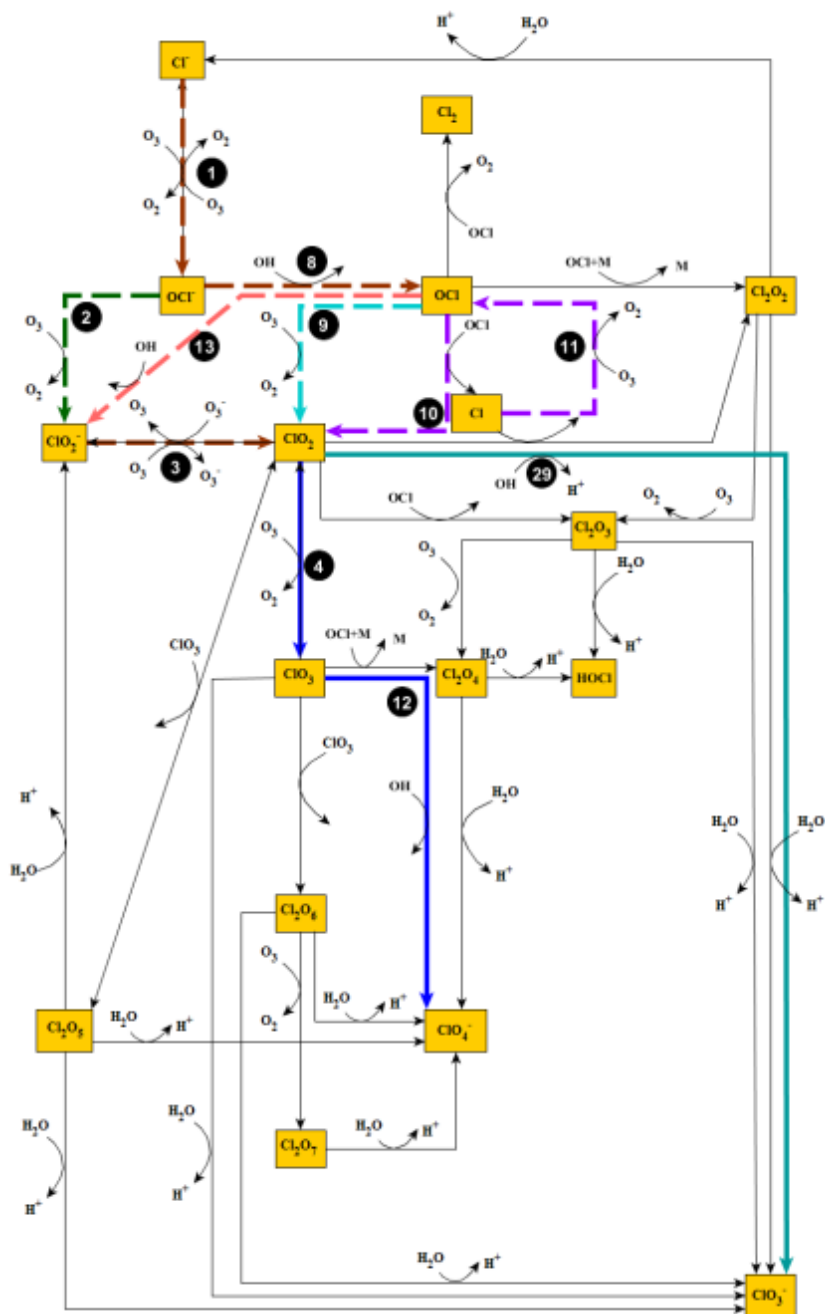


**Figure 2.5.17. Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{Cl}^-$  including possible pathways for  $\text{ClO}_3^-$  formation (aq).** Note the thick dashed lines indicate the initial reactions that are possibly shared between  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  pathways and the thick aqua lines represent the final reactions leading to  $\text{ClO}_3^-$  formation. Both  $\text{ClO}_3^-$  pathways lead to the formation of one  $\text{ClO}_3^-$  molecule containing one O from  $\text{O}_3$ .

### ***O<sub>3</sub>-Aqueous Chloride***

There are at least twenty-six possible pathways that could lead to the formation of ClO<sub>4</sub><sup>-</sup> from O<sub>3</sub> oxidation of Cl<sup>-</sup> (aq) (Appendix A: Figures A.1–A.26). Four speculated initial reaction sequences: [1] Rxn's 1– 3; [2] Rxn's 1, 8 – 9; [3] Rxn's 1, 8, 10 – 11; and [4] Rxn's 1, 3, 8, 13 and five final reaction sequences: [1] Rxn's 4 – 7; [2] Rxn's 4 and 12; [3] Rxn's 4 – 5 and 14; [4] Rxn's 4, 22, and 17; and [5] Rxn's 15 – 17 and Rxn's 20 – 21 were identified (Appendix A Figures A.1–A.26; Table 2.5.4). All pathways produced ClO<sub>2</sub> (aq) as an intermediate.

Our ClO<sub>4</sub><sup>-</sup>Δ<sup>17</sup>O values (+29 ‰ to + 32 ‰) suggest that the O<sub>3</sub> oxidation of Cl<sup>-</sup> (aq) includes intermediate reactions that will lead to the formation of a ClO<sub>4</sub><sup>-</sup> molecule with all or nearly all (depending on the final site preference value) O originating from O<sub>3</sub>; therefore, any mechanisms resulting in a ClO<sub>4</sub><sup>-</sup> with less than four O from O<sub>3</sub> 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 O<sub>3</sub> lowers the number of possible mechanisms from twenty-six to only eight, all of which require the formation of both ClO<sub>2</sub> (aq) and the ClO<sub>3</sub> 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 ClO<sub>3</sub> radical with an OH radical and four of them (Appendix A: Figures A.5 – A.8) require the formation of the Cl<sub>2</sub>O<sub>7</sub> species. The Cl<sub>2</sub>O<sub>7</sub> species hydrolyzes to form two ClO<sub>4</sub><sup>-</sup> molecules (Table 2.5.4; Rxn 7), one containing three and the other four O from O<sub>3</sub>. It has been speculated that Cl<sub>2</sub>O<sub>7</sub> is involved in the production of ClO<sub>4</sub><sup>-</sup>, but our ClO<sub>4</sub><sup>-</sup>-Δ<sup>17</sup>O values indicate that this may not be the case as only one of the two ClO<sub>4</sub><sup>-</sup> molecules formed from hydrolysis of this intermediate species has a structure in which all O's originate from O<sub>3</sub>. However, the unknown degree of site preference can allow for a limited amount of ClO<sub>4</sub><sup>-</sup> with only three O from O<sub>3</sub> and thus it is possible that a mixture of the two ClO<sub>4</sub><sup>-</sup> molecules produced from hydrolysis of the Cl<sub>2</sub>O<sub>7</sub> species is in part responsible for the ClO<sub>4</sub><sup>-</sup> formed in our Cl<sup>-</sup> (aq) experiments. It might be unlikely that reactions involving the Cl<sub>2</sub>O<sub>7</sub> species are responsible for substantial ClO<sub>4</sub><sup>-</sup> production, unless unknown Cl<sub>2</sub>O<sub>7</sub> reaction pathways exist that include transfer of an additional O from O<sub>3</sub> (e.g. O radical) rather than from water. However, given the high Δ<sup>17</sup>O value of ClO<sub>4</sub><sup>-</sup> observed, it is possible that ClO<sub>4</sub><sup>-</sup> is a result of mixing between branching pathways involving the OH radical and Cl<sub>2</sub>O<sub>7</sub> species.

Elimination of the pathways involving the Cl<sub>2</sub>O<sub>7</sub> species would reduce the number of plausible mechanisms responsible for ClO<sub>4</sub><sup>-</sup> formation by O<sub>3</sub> oxidation of Cl<sup>-</sup> (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 ClO<sub>3</sub> 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 O<sub>3</sub> or has another origin. Our interpretation of the ClO<sub>4</sub><sup>-</sup> Δ<sup>17</sup>O values along with the evaluation of the proposed pathways suggest that ClO<sub>4</sub><sup>-</sup> with four O from O<sub>3</sub> is possibly mainly formed through a final oxidation reaction involving an OH radical and not through the Cl<sub>2</sub>O<sub>7</sub>

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 O<sub>3</sub>.

The  $\Delta^{17}\text{O}$  values of  $\text{ClO}_3^-$  produced during  $\text{ClO}_4^-$  production from O<sub>3</sub> oxidation of  $\text{Cl}^-$  (aq) indicate that only one O in  $\text{ClO}_3^-$  could be derived from an O<sub>3</sub> molecule. This could occur through reactions that lead to the incorporation of a single O from O<sub>3</sub> or through a mixture of pathways that lead to zero to four O being incorporated from O<sub>3</sub>. If  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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 O<sub>3</sub> to the  $\text{ClO}_3^-$  molecules, with the final  $\text{ClO}_3^-$  containing anywhere from two to three O from O<sub>3</sub>. Even pathways only sharing two of the initial reactions (Rxn's 1 and 8) would result in a  $\text{ClO}_3^-$  with two O from O<sub>3</sub> (Figure 2.5.18). In addition, none of the pathways that we identified as possible  $\text{ClO}_4^-$  formation mechanisms (Figure 2.5.16) produced  $\text{ClO}_3^-$  directly from the production of  $\text{ClO}_4^-$ , further indicating the independence of  $\text{ClO}_4^-$  from  $\text{ClO}_3^-$  pathways. A couple of factors could be responsible for  $\text{ClO}_3^-$  only having one O from O<sub>3</sub> including: (1) non O<sub>3</sub> related reactions that have not yet been identified and (2) oxygen exchange during equilibrium reactions. In support of the likelihood of independent  $\text{ClO}_3^-$  production pathways,  $\text{ClO}_3^-/\text{ClO}_4^-$  molar ratios (3000–4400) are orders of magnitude higher than for dry oxidation of  $\text{Cl}^-$  even though  $\text{ClO}_4^-$  production was relatively similar. Based on the ratios we would expect that the major reactions lead to  $\text{ClO}_3^-$  formation and if so, based on our  $\text{ClO}_3^-$   $\Delta^{17}\text{O}$  values, we could speculate that the major source of oxidation for formation of  $\text{ClO}_3^-$  is not O<sub>3</sub>, but rather another species. Given our relatively high  $\text{ClO}_3^-/\text{ClO}_4^-$  molar ratios in our  $\text{Cl}^-$  (aq) experiments compared to those of O<sub>3</sub> oxidation of dry  $\text{Cl}^-$ , it is evident that hydrolysis reactions are not as important for  $\text{ClO}_4^-$  formation as they are for  $\text{ClO}_3^-$ . Our  $\text{ClO}_3^-/\text{ClO}_4^-$  ratios in conjunction with our  $\text{ClO}_4^-$   $\Delta^{17}\text{O}$  values further suggest and support our theory that the involvement of O<sub>3</sub> in  $\text{ClO}_4^-$  formation mechanisms due to oxidation of  $\text{Cl}^-$  is not hindered by the presence of H<sub>2</sub>O, but rather that H<sub>2</sub>O may not be required for formation of  $\text{ClO}_4^-$  to occur.

It may be possible to determine the feasibility of the  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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  $\text{OCl}^-$  species that when oxidized can either form  $\text{ClO}_2^-$  or the  $\text{OCl}$  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  $\text{OCl}$  radical by means of an OH radical, whereas the slower reaction leads to formation of  $\text{ClO}_2^-$  by means of the O<sub>3</sub> molecule (Rxn's 8 and 2, respectively). Compared to other  $\text{ClO}_x$  species,  $\text{Cl}^-$  (dry and aqueous) reacted slowly and O<sub>3</sub> was unlimited, giving way for more reactions with O<sub>3</sub> to occur. Reactions 2 and 8 (Table 2.5.4) both imply that O<sub>3</sub> reactions occur much slower than reactions involving a non O<sub>3</sub> species and thus because major reactions are expected to form  $\text{ClO}_3^-$ , it is likely that pathways leading to  $\text{ClO}_3^-$  follow Reaction 8 (Table

2.5.4), which involves the OH radical, and pathways leading to  $\text{ClO}_4^-$  follow Reaction 2 (Table 2.5.4), which involves the  $\text{O}_3$  molecule. The reactions for the decomposition of the  $\text{ClO}_2$  species also support  $\text{ClO}_4^-$  being formed by slow reactions involving  $\text{O}_3$ . In the  $\text{ClO}_4^-$  pathways identified (Figure 2.5.16), we propose that  $\text{ClO}_2$  is oxidized to the  $\text{ClO}_3$  radical by  $\text{O}_3$  (Table 2.5.4; Rxn 4). This reaction is much slower compared to reactions that either lead to the formation of the  $\text{Cl}_2\text{O}_2$  species (Table 2.5.4; Rxn 18), the formation of  $\text{ClO}_3^-$  (Table 2.5.4; Rxn 29), or the formation of the  $\text{Cl}_2\text{O}_3$  species, indicating that the pathways we propose may well be the pathways responsible for  $\text{ClO}_4^-$  formation in this study.

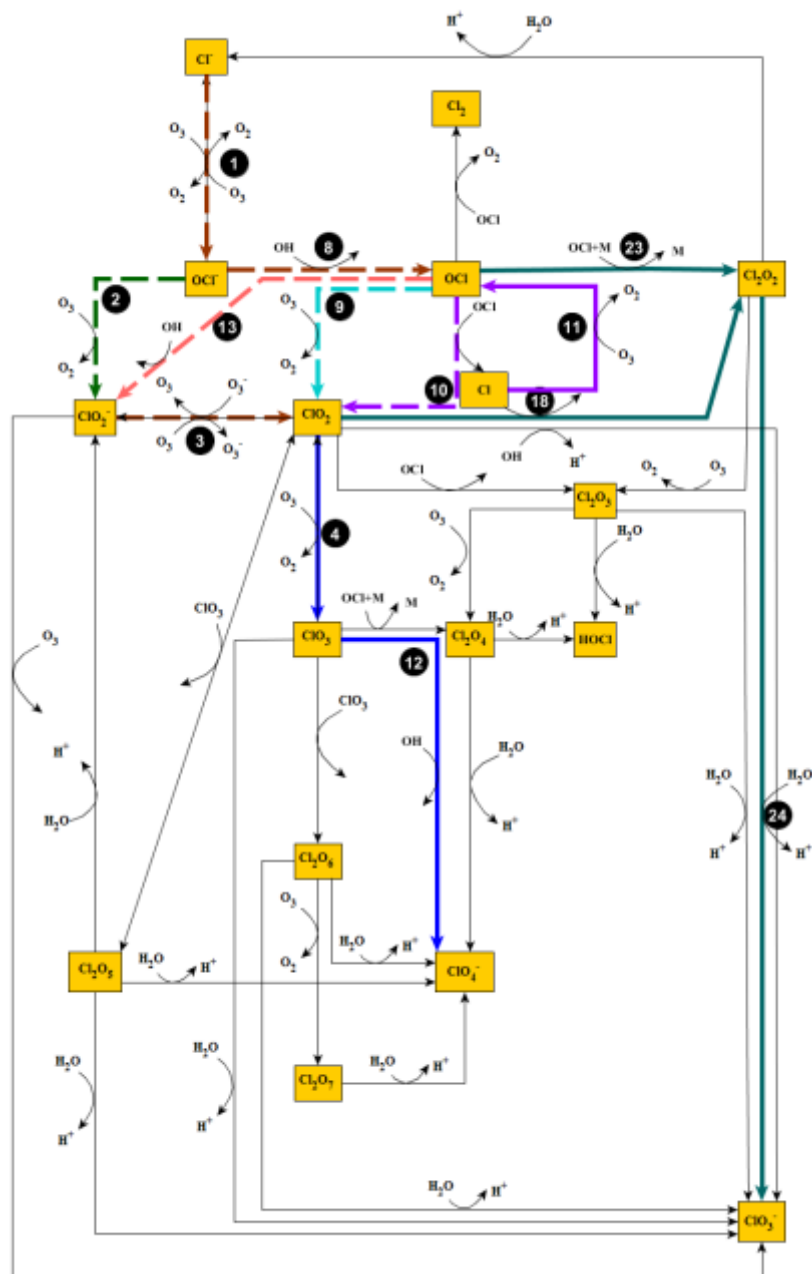


**Figure 2.5.18. Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq) including possible pathways for  $\text{ClO}_3^-$  formation.** Note the thick dashed lines indicate the initial reactions that are possibly shared between  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  pathways and the thick aqua lines represent the final reactions leading to  $\text{ClO}_3^-$  formation. Both  $\text{ClO}_3^-$  pathways lead to the formation of one  $\text{ClO}_3^-$  molecule containing one O from  $\text{O}_3$ .

### ***O<sub>3</sub>-Hypochlorite***

With the exception of reaction 1 (Table 2.5.4), all mechanisms possibly leading to ClO<sub>4</sub><sup>-</sup> production from O<sub>3</sub> oxidation of OCl<sup>-</sup> (aq) (Appendix: Figures A.27 – A.49) were the same as the mechanisms identified leading to ClO<sub>4</sub><sup>-</sup> formation from O<sub>3</sub> oxidation of Cl<sup>-</sup> (aq). For further evaluation, these twenty-six mechanisms were divided into five groups: [1] pathways leading to ClO<sub>4</sub><sup>-</sup> with only three O atoms from O<sub>3</sub> (Appendix: Figures A.27 – A.30), [2] pathways leading to production of two ClO<sub>4</sub><sup>-</sup> molecules containing two and three O atoms from O<sub>3</sub>, respectively (Appendix: Figures A.31 – A.34), [3] pathways leading to ClO<sub>4</sub><sup>-</sup> with only two O atoms from O<sub>3</sub> (Appendix: Figures A.35 – A.39), [4] pathways involving the formation of the Cl<sub>2</sub>O<sub>5</sub> intermediate species (Appendix: Figures A.40 – A.43), and [5] pathways involving reactions 16 and 19, reactions involving the oxidation of Cl<sub>2</sub>O<sub>3</sub> into Cl<sub>2</sub>O<sub>4</sub> and oxidation of Cl<sub>2</sub>O<sub>2</sub> into Cl<sub>2</sub>O<sub>3</sub> by O<sub>3</sub>, respectively (Appendix: Figures A.44 – A.49). Assuming that the Δ<sup>17</sup>O values (+29 ‰ to +33‰) of ClO<sub>4</sub><sup>-</sup> from the O<sub>3</sub>-Cl<sup>-</sup> (dry and aqueous) experiments can be used as a reference to determine site preference and reaction selectivity of terminal O atoms from O<sub>3</sub> into ClO<sub>4</sub><sup>-</sup> we would expect that each O from O<sub>3</sub> would contribute about 8 ‰. Based on this assumption, our ClO<sub>4</sub><sup>-</sup>-Δ<sup>17</sup>O values produced from OCl<sup>-</sup> (+ 16 ‰ to + 20 ‰) suggest that the O<sub>3</sub> oxidation of OCl<sup>-</sup> (aq) results in a ClO<sub>4</sub><sup>-</sup> molecule with at least two but less than three O from O<sub>3</sub>, thus we were unable to eliminate pathways based on the number of O atoms derived from O<sub>3</sub> as the Δ<sup>17</sup>O values of our ClO<sub>4</sub><sup>-</sup> could be the result of a combination of pathways yielding ClO<sub>4</sub><sup>-</sup> having zero to four O from O<sub>3</sub>. Furthermore, OCl<sup>-</sup> is a direct product of the reaction between Cl<sup>-</sup> and O<sub>3</sub> (Table 2.5.4: Rxn 1), thus it is also possible that OCl<sup>-</sup> and Cl<sup>-</sup> share the same pathway leading to the production of ClO<sub>4</sub><sup>-</sup>. 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<sub>2</sub>O<sub>5</sub> species has two possible isomers (Table 2.5.4) and only one isomer will lead to the formation of ClO<sub>4</sub><sup>-</sup> (Quiroga et al., 2005). Due to the lack of knowledge regarding the isomer structures of Cl<sub>2</sub>O<sub>5</sub> and the likeliness of a mechanism forming ClO<sub>3</sub><sup>-</sup> from the Cl<sub>2</sub>O<sub>5</sub> species instead, all mechanisms involving Cl<sub>2</sub>O<sub>5</sub> were regarded as being unlikely to account for ClO<sub>4</sub><sup>-</sup> 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<sub>4</sub><sup>-</sup> from the reaction between OCl and ClO<sub>3</sub> 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<sub>4</sub><sup>-</sup> in our study, nine more than those identified for ClO<sub>4</sub><sup>-</sup> formed from the O<sub>3</sub> oxidation of dry Cl<sup>-</sup> (simultaneously represented in Figure 2.5.16). Similarly to O<sub>3</sub> oxidation of Cl<sup>-</sup> (aq) the remaining pathways all require the formation of ClO<sub>2</sub> (aq) and a subsequent ClO<sub>3</sub> 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  $\text{Cl}^-$  versus that of  $\text{OCl}^-$  in the presence of  $\text{O}_3$ . Complete decay of  $\text{OCl}^-$  happened in hours compared to  $\text{Cl}^-$  loss which took months and did not fully complete. Molar yields of  $\text{ClO}_4^-$  for the  $\text{O}_3$ - $\text{OCl}^-$  (0.01- 0.07 %) experiments were at least an order of magnitude higher than the molar yields of  $\text{ClO}_4^-$  from the  $\text{O}_3$ - $\text{Cl}^-$  (aq) experiments (< 0.001 %) even though the initial mass of  $\text{Cl}^-$  was two to three orders of magnitude higher than the initial molar mass of  $\text{OCl}^-$  (Table 2.5.3). Although the molar mass of transformed  $\text{Cl}^-$  was less (1.5 %) compared to transformed  $\text{OCl}^-$  (99.98 %), in the  $\text{Cl}^-$  experiments there was an excess amount of  $\text{Cl}^-$  and  $\text{O}_3$  in solution as  $\text{O}_3$  was being continuously input into the system, allowing for continuous reactions between  $\text{Cl}^-$  and  $\text{O}_3$  to occur. Yet the rate of reaction between  $\text{Cl}^-$  and  $\text{O}_3$  might have been much slower compared to the rate of reactions between  $\text{Cl}^-$  and other species. Due to the rate of reaction and input rate of  $\text{O}_3$  for the oxidation of  $\text{OCl}^-$ ,  $\text{O}_3$  was likely limiting, perhaps facilitating reactions not involving  $\text{O}_3$  and thus allowing alternate reactions (e.g. Rxn's 7, 14, 22, and 17) not observed in  $\text{O}_3$  oxidation of  $\text{Cl}^-$  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  $\text{ClO}_4^-$  to form from  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Based on this data, it is assumed that  $\text{ClO}_4^-$  production from  $\text{O}_3$  oxidation of  $\text{OCl}^-$  is not entirely dependent on the same pathway as that of  $\text{ClO}_4^-$  from  $\text{O}_3$  oxidation of  $\text{Cl}^-$ , but is rather a mixture of that pathway and other pathways not entirely dependent on  $\text{O}_3$  or  $\text{O}_3$  related oxidant reactions.

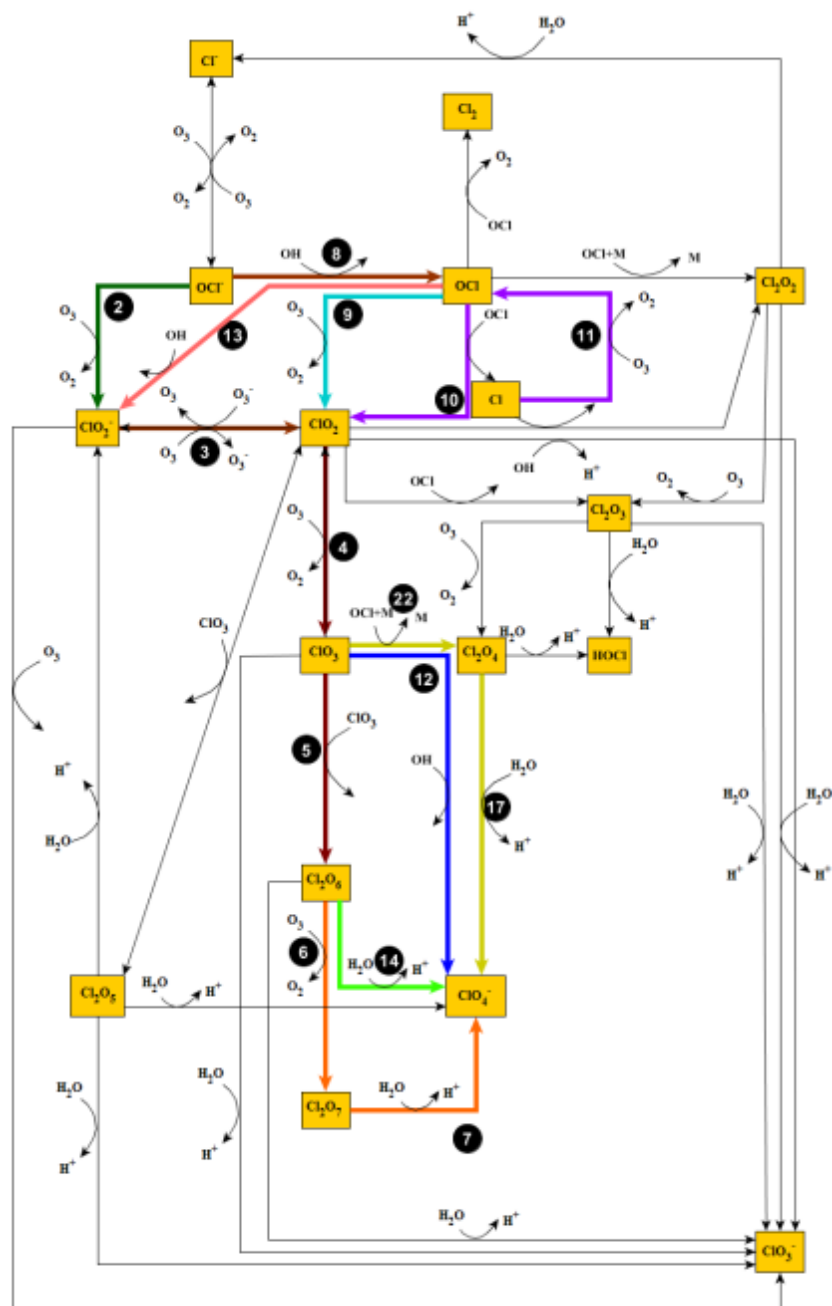


**Figure 2.5.19. Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq).** Note the thick forest dark red lines indicate the reactions that some of the species may share while other colored lines represent reactions unique to each of the proposed pathways. All pathways lead to the formation of a  $\text{ClO}_4^-$  molecule containing either two or three O's from  $\text{O}_3$ .

Our results for the  $\text{OCl}^-$  experiments further support the theory that formation of  $\text{ClO}_3^-$  may be independent of formation of  $\text{ClO}_4^-$ .  $\Delta^{17}\text{O}$  values for  $\text{ClO}_3^-$  (-0.02 ‰ to + 1.37 ‰) indicate that the O in  $\text{ClO}_3^-$  is not primarily derived from  $\text{O}_3$  (+ 24.5 ‰). 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  $\text{ClO}_3^-$  molecule along with  $\text{ClO}_4^-$ . These pathways would produce a  $\text{ClO}_3^-$  with two O atoms derived from  $\text{O}_3$  and thus, cannot be responsible for  $\text{ClO}_3^-$  formation in our study. Three pathways (Figure 2.5.20), independent of  $\text{ClO}_4^-$  formation, were determined to be possible for formation of a  $\text{ClO}_3^-$  molecule with no O from  $\text{O}_3$ . Given that reaction sequences that would form  $\text{ClO}_3^-$  during  $\text{O}_3$  oxidation of  $\text{Cl}^-$  would form  $\text{ClO}_3^-$  with two O from  $\text{O}_3$  (Figure 2.5.18), the  $\text{ClO}_3^-$  formed from  $\text{O}_3$  oxidation of  $\text{OCl}^-$  is consistent as reaction 1 (Table 2.5.4) would be eliminated reducing the contribution of O from  $\text{O}_3$  to near zero.

Final  $\text{ClO}_3^-/\text{ClO}_4^-$  product ratios for  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq) (482-527) were not as high as those for  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). These molar ratios indicate a higher production of  $\text{ClO}_3^-$  compared to  $\text{ClO}_4^-$ , supporting our hypothesis that  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{OCl}^-$  may be independent of that of  $\text{ClO}_3^-$ . However, our product yields indicate that a significant amount of  $\text{OCl}^-$  is transformed to  $\text{Cl}^-$ , not  $\text{ClO}_3^-$  or  $\text{ClO}_4^-$ , suggesting that the main pathway rather leads to  $\text{Cl}^-$  not  $\text{ClO}_3^-$  (Table 2.5.3). This pathway may be the result of reaction 1 (Table 2.5.4), a back reaction involving the conversion of  $\text{OCl}^-$  to  $\text{Cl}^-$ .

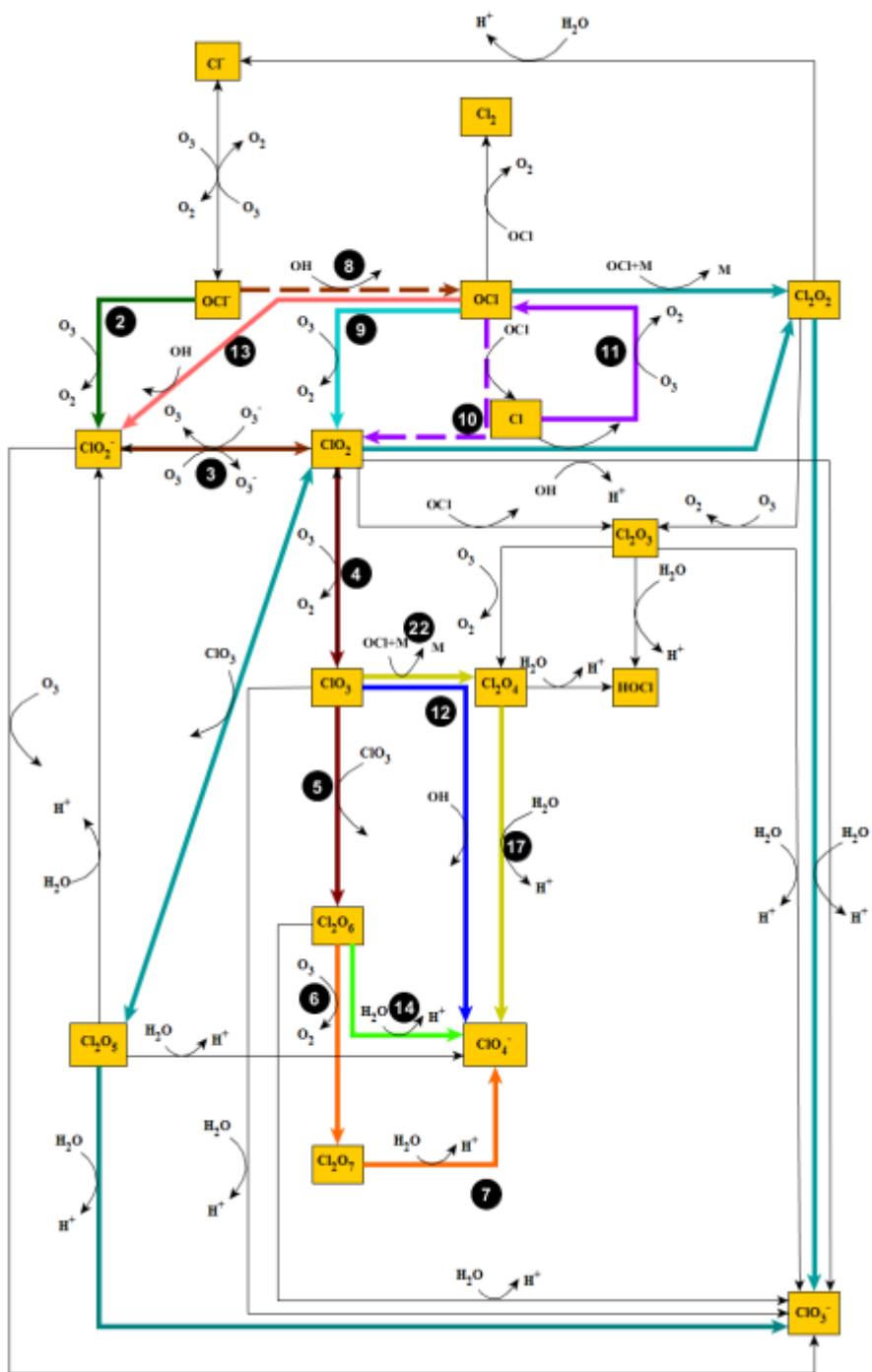


**Figure 2.5.20. Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{OCl}^-$  including possible pathways for  $\text{ClO}_3^-$  formation (aq).** Note the thick dashed lines indicate the initial reactions that are possibly shared between  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  pathways and the thick aqua lines represent the final reactions leading to  $\text{ClO}_3^-$  formation.

### ***O<sub>3</sub>-Chlorite***

ClO<sub>4</sub><sup>-</sup> produced from O<sub>3</sub> oxidation of ClO<sub>2</sub><sup>-</sup> ( $\Delta^{17}\text{O} = +12\text{‰}$  to  $+13\text{‰}$ ) had a lower  $\Delta^{17}\text{O}$  than ClO<sub>4</sub><sup>-</sup> produced from OCl<sup>-</sup> or Cl<sup>-</sup>. Using the  $\Delta^{17}\text{O}$  values for ClO<sub>4</sub><sup>-</sup> produced from O<sub>3</sub> oxidation of Cl<sup>-</sup> (aq) ( $+29\text{‰}$  to  $+32\text{‰}$ ), for which all four O must come from O<sub>3</sub>, as a reasonable approximation of the site preference value, we would expect that ClO<sub>4</sub><sup>-</sup> incorporating two O from O<sub>3</sub> would have a  $\Delta^{17}\text{O}$  value near the ranges of  $+14.5\text{‰}$  to  $+16\text{‰}$ . This suggests that O<sub>3</sub> oxidation of ClO<sub>2</sub><sup>-</sup> results in the transfer of two or less O from O<sub>3</sub>.

A total of six mechanisms were postulated to form ClO<sub>4</sub><sup>-</sup> from O<sub>3</sub> oxidation of ClO<sub>2</sub><sup>-</sup> (aq) (Appendix A: Figures A.50 – A.55). All pathways starting from ClO<sub>2</sub><sup>-</sup> produce ClO<sub>4</sub><sup>-</sup> with one or two O from O<sub>3</sub>. Two pathways (Appendix A: Figures A.54 and A.55) were eliminated because they either required formation of the Cl<sub>2</sub>O<sub>5</sub> species or the formation of the Cl<sub>2</sub>O<sub>4</sub> species by the oxidation of Cl<sub>2</sub>O<sub>3</sub> by O<sub>3</sub> (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 ClO<sub>4</sub><sup>-</sup> with one O from O<sub>3</sub> and two (Appendix A: Figures A.50 – A.51) produce a ClO<sub>4</sub><sup>-</sup> with two O's from O<sub>3</sub> (similar to that indicated by the  $\Delta^{17}\text{O}$  values of ClO<sub>4</sub><sup>-</sup>).

The pathways (Appendix A: Figures A.50 – A.51) leading to ClO<sub>4</sub><sup>-</sup> with two O from O<sub>3</sub> are consistent with the pathways observed for O<sub>3</sub> oxidation of Cl<sup>-</sup> (aq) and OCl<sup>-</sup> given the consistent decrease in  $\Delta^{17}\text{O}$  of ClO<sub>4</sub><sup>-</sup> indicating a loss of O transfer from each reaction step starting with O<sub>3</sub> oxidation of Cl<sup>-</sup> (aq). In the Cl<sup>-</sup> (aq) pathway, the first reaction donates an O to Cl<sup>-</sup> from O<sub>3</sub> (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 O<sub>3</sub>, to OCl<sup>-</sup> to form ClO<sub>2</sub><sup>-</sup>.

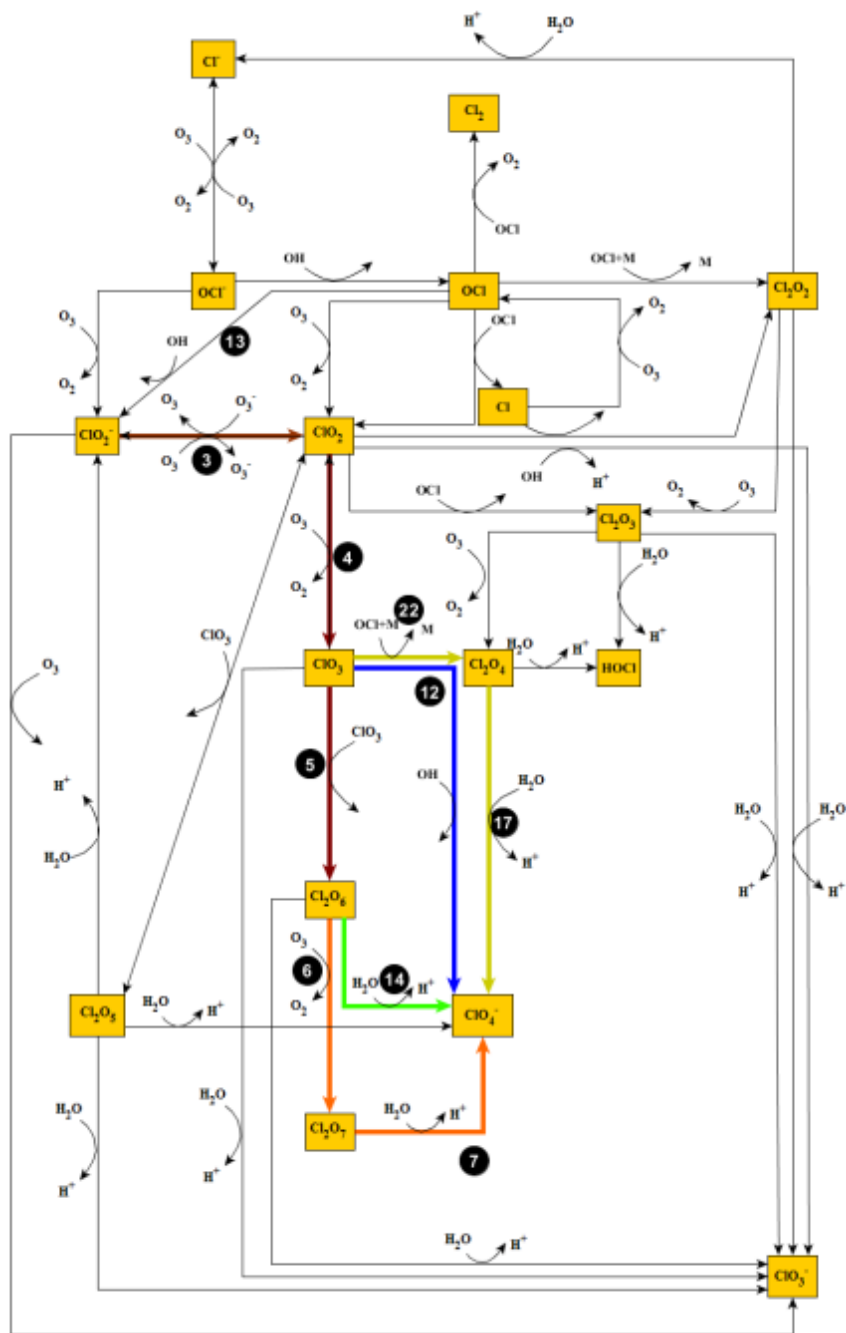


**Figure 2.5.21. Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  (aq).** Note the thick forest dark red lines indicate the reactions that some of the species may share while other colored lines represent reactions unique to each of the proposed pathways. All pathways lead to the formation of a  $\text{ClO}_4^-$  molecule containing either one or two O's from  $\text{O}_3$ .

$\text{ClO}_3^-$  was the major product of the  $\text{O}_3\text{-ClO}_2^-$  experiments and in accordance with the previous  $\text{ClO}_3^-$ - $\Delta^{17}\text{O}$  values of the  $\text{Cl}^-$  (aq) and  $\text{OCl}^-$  (aq) experiments,  $\Delta^{17}\text{O}$  values (+ 3 ‰ to + 4 ‰) of  $\text{ClO}_3^-$  formed from  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  suggest that  $\text{ClO}_3^-$  formation pathways may be independent of those of  $\text{ClO}_4^-$  as only about half of one O in  $\text{ClO}_3^-$  is derived from  $\text{O}_3$ . This is further supported by the  $\text{ClO}_3^-/\text{ClO}_4^-$  molar ratios (902 – 1111) which indicate that the main pathway would lead to  $\text{ClO}_3^-$  formation and the minor pathways to  $\text{ClO}_4^-$ . Four pathways (Figure 2.5.22) were proposed as possibly leading to the  $\text{ClO}_3^-$  produced in our  $\text{ClO}_2^-$  (aq) experiments. Three pathways share the initial reaction (Reaction 3: formation of  $\text{ClO}_2$  (aq)) with the  $\text{ClO}_4^-$  pathways, but then diverge into separate reaction sequences to produce  $\text{ClO}_3^-$  (Figure 2.5.22). All reactions proposed to occur after reaction 3 that lead to  $\text{ClO}_3^-$  formation are fast (Rxn's 15 and 29; Table 3.4) compared to reaction 4, which leads to  $\text{ClO}_4^-$  formation (Figure 2.5.22; Table 2.5.4), indicating that our proposed mechanisms for  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  formation might be correct. Out of the four  $\text{ClO}_3^-$  pathways proposed, two of the pathways (those involving Rxn's 26 and 27; Table 2.5.4 and Figure 2.5.22) form a  $\text{ClO}_3^-$  molecule with one O from  $\text{O}_3$  and the other two pathways (those involving Rxn's 20 – 21 and 29; Table 2.5.4; Figure 2.5.22) form a  $\text{ClO}_3^-$  molecule with possibly zero O from  $\text{O}_3$  depending on where the O from  $\text{O}_3$  is located in the  $\text{Cl}_2\text{O}_5$  structure and whether the O in the OH radical is derived from the  $\text{O}_3$  molecule. These pathways suggest that  $\text{ClO}_3^-$  formed from  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  may be the result of a mixture of different formation mechanisms (Table 2.5.4).

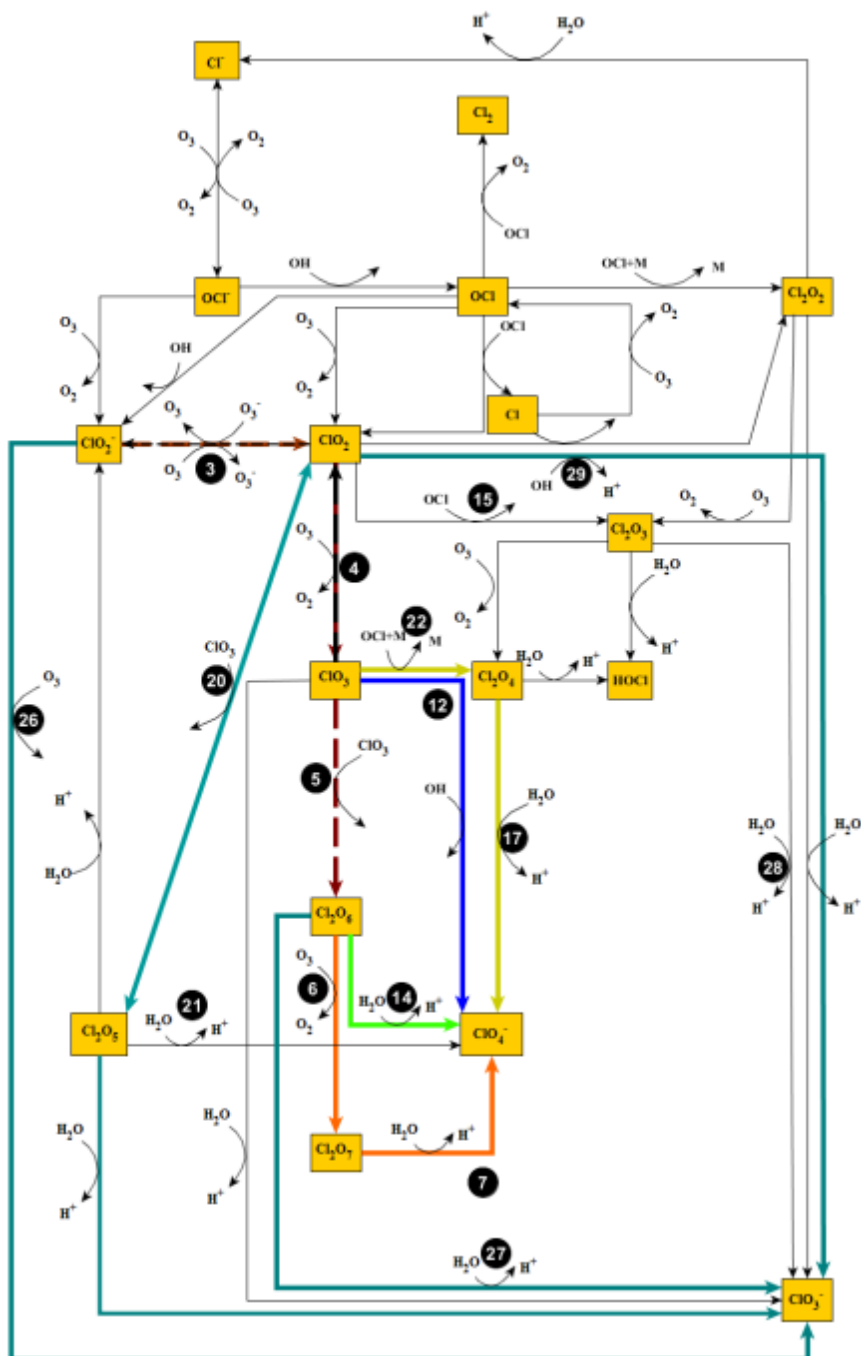


**Figure 2.5.22. Combined final pathways determined responsible for  $\text{ClO}_4^-$  formation by  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  including possible pathways for  $\text{ClO}_3^-$  formation (aq).** Note the thick dashed lines indicate the initial reactions that are possibly shared between  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  pathways and the thick aqua lines represent the final reactions leading to  $\text{ClO}_3^-$  formation.



### ***O<sub>3</sub>-Chlorine Dioxide***

The proposed mechanisms for the formation of ClO<sub>4</sub><sup>-</sup> from molecular O<sub>3</sub> and ClO<sub>2</sub> (aq) are summarized in Appendix A Figures A.56 – A.61 and either produce a ClO<sub>4</sub><sup>-</sup> with one or two O from O<sub>3</sub>. ClO<sub>4</sub><sup>-</sup> produced from O<sub>3</sub> oxidation of ClO<sub>2</sub> (aq) had Δ<sup>17</sup>O values (+16 ‰ to + 17 ‰) similar, yet slightly less than oxidation of OCl<sup>-</sup> and higher than oxidation of ClO<sub>2</sub><sup>-</sup>. Assuming our estimated value for the site preference are correct based on ClO<sub>4</sub><sup>-</sup> produced from O<sub>3</sub> oxidation of Cl<sup>-</sup> (e.g. O ≈ 8 ‰), all pathways producing a ClO<sub>4</sub><sup>-</sup> with less than two O from O<sub>3</sub> were eliminated as possible ClO<sub>4</sub><sup>-</sup> 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 Δ<sup>17</sup>O value for ClO<sub>4</sub><sup>-</sup> produced from ClO<sub>2</sub> (aq) compared to ClO<sub>2</sub><sup>-</sup> is unclear but perhaps is due to variation in the site preference value. The transformation of ClO<sub>2</sub><sup>-</sup> to ClO<sub>2</sub> (aq) does not involve an O transfer. Perhaps the much higher concentration (60 – 69 mmol) of reactant in the ClO<sub>2</sub><sup>-</sup> oxidation experiment compared to ClO<sub>2</sub> (aq) (0.5 – 1.1 mmol) experiment produced an effect on the site preference value. The oxidation of ClO<sub>2</sub><sup>-</sup> (rapid reaction) may have resulted in O<sub>3</sub> limitations, while for ClO<sub>2</sub> (aq) oxidation (also rapid) O<sub>3</sub> may not have been limiting due to the lower reactant concentration. The yield of ClO<sub>4</sub><sup>-</sup> for both ClO<sub>2</sub> (aq) and ClO<sub>2</sub><sup>-</sup> were the highest for all experiments although they were twenty to thirty fold higher for ClO<sub>2</sub> (aq) than ClO<sub>2</sub><sup>-</sup>.

The ClO<sub>3</sub><sup>-</sup>/ClO<sub>4</sub><sup>-</sup> molar ratios (21-30) for O<sub>3</sub> oxidation of ClO<sub>2</sub> (aq) were lower compared to those of the ClO<sub>2</sub><sup>-</sup> (aq) and even the Cl<sup>-</sup> (aq) (3022 – 4431) and OCl<sup>-</sup> (482 – 569) experiments, supporting the previous postulations that ClO<sub>3</sub><sup>-</sup> production is due to reactions unrelated to ClO<sub>4</sub><sup>-</sup> production and that major ClO<sub>3</sub><sup>-</sup> pathways may diverge from those of ClO<sub>4</sub><sup>-</sup> early in the reaction sequence. Aside from ClO<sub>4</sub><sup>-</sup>, ClO<sub>3</sub><sup>-</sup> was the only other species produced in the ClO<sub>2</sub> (aq) experiments, but without isotopic data for ClO<sub>3</sub><sup>-</sup>, we were unable to determine the mechanisms involved in ClO<sub>3</sub><sup>-</sup> formation.

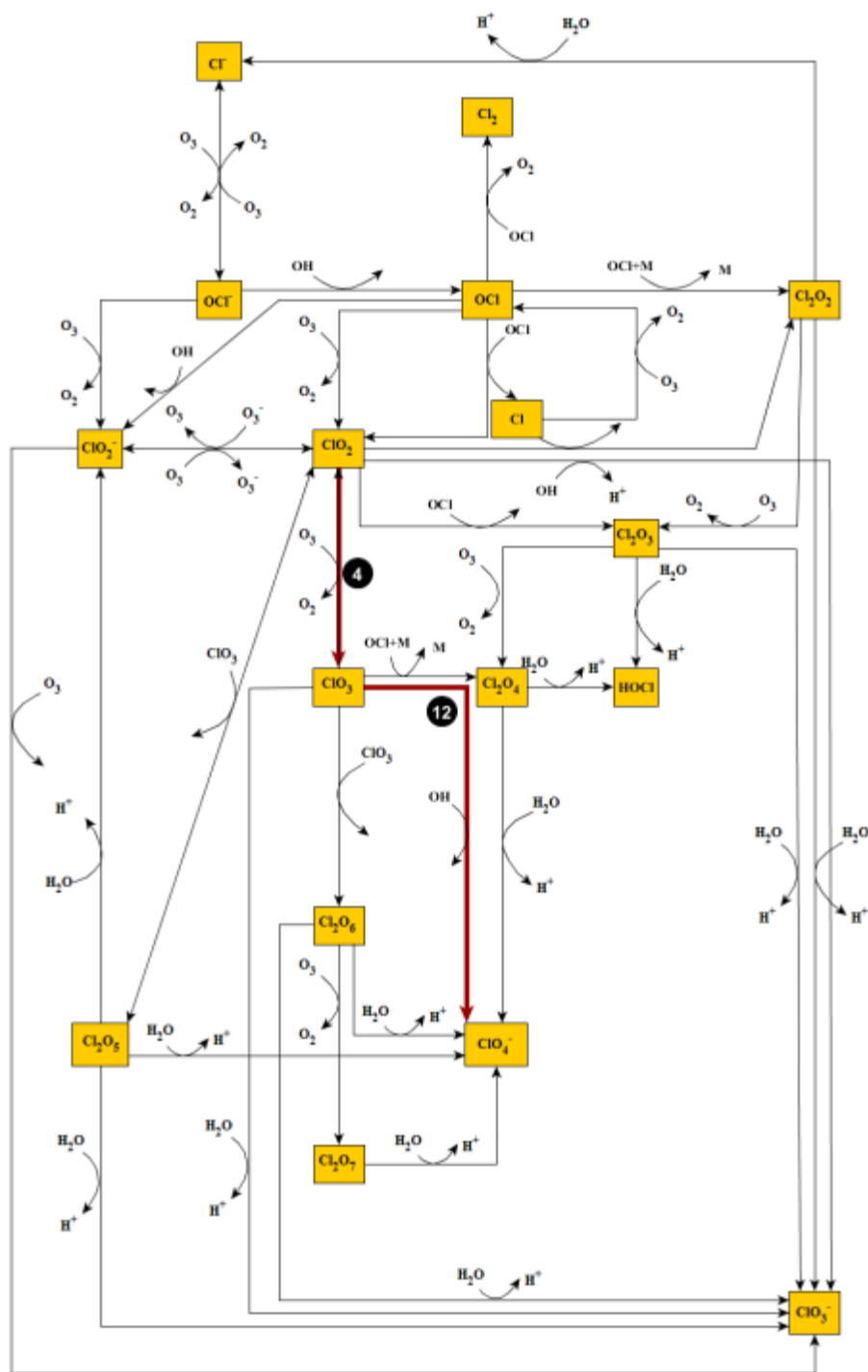


**Figure 2.5.23.  $\text{O}_3$  oxidation of  $\text{ClO}_2$  (aq). Note the thick maroon lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 2.5.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing two O from  $\text{O}_3$ .**

#### 2.5.4.10 Overall Conclusions Based on $\Delta^{17}\text{O}$ of $\text{O}_3$ Experiments

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

The  $\Delta^{17}\text{O}$  values of  $\text{ClO}_3^-$  compared to those of  $\text{ClO}_4^-$  also indicate that  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$  are formed through independent pathways.  $\text{ClO}_3^-$  was the main species produced, indicating that major reactions of  $\text{O}_3$  with  $\text{ClO}_x$  produce  $\text{ClO}_3^-$ , not  $\text{ClO}_4^-$ . Although not all reaction rates for reactions in our proposed pathways are known, there are reaction rates for the decay of  $\text{OCl}^-$  and  $\text{ClO}_2(\text{aq})$  (Rxn's 2, 4, 8, 15, 18, and 29; Table 2.5.4) that indicated that the slower reaction (Rxn 4, Table 2.5.4) is the one likely responsible for  $\text{ClO}_4^-$  formation.

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

##### **UV-Hypochlorite**

The reactions involved in the photodecomposition of  $\text{OCl}^-$  are well-known and possible  $\text{ClO}_4^-$  formation mechanisms have been proposed from them (Kang et al., 2006; Buxton et al., 1972a,b). These  $\text{ClO}_4^-$  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  $\text{OCl}^-$  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  $\text{OCl}^-$ , 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  $\text{O}(^3\text{P})$  atom to oxidize  $\text{OCl}^-$ .

The low  $\Delta^{17}\text{O}$  values (- 0.0 ‰ and + 0.5 ‰) of  $\text{ClO}_4^-$  formed from UV oxidation of  $\text{OCl}^-$  indicated no involvement of  $\text{O}_3$  and thus, it was determined that pathways leading to  $\text{ClO}_4^-$  did not form  $\text{O}_3$  and therefore, could not be postulated on the basis of the number of O atoms coming from the  $\text{O}_3$  molecule. However, because UV experiments were conducted at a wavelength of 350 nm, the pathways most likely involved in production of  $\text{ClO}_4^-$  are believed to be the two

involving the oxidation of  $\text{OCl}^-$  by the ground state  $\text{O}(^3\text{P})$  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  $\text{O}_3$  oxidation of  $\text{OCl}^-$ , the formation of two key intermediate species was found necessary for  $\text{ClO}_4^-$  production in the UV experiments:  $\text{ClO}_2$  and the  $\text{ClO}_3$  radical (Figure 2.5.24). The only uncertainty associated with the two  $\text{O}(^3\text{P})$ - $\text{ClO}_4^-$  pathways is as to which reactions are responsible for the formation of  $\text{ClO}_2$ : Rxn's 17 and 21 or Rxn 20 (Figure 2.5.24). The reactions following  $\text{ClO}_2$  formation, which included oxidation of  $\text{ClO}_2$  into the  $\text{ClO}_3$  radical (Rxn's 30 and 41), the reaction between two  $\text{ClO}_3$  radicals to produce  $\text{Cl}_2\text{O}_6$  (Rxn 42), and the hydrolysis of the  $\text{Cl}_2\text{O}_6$  species (Rxn 43) were common and deemed necessary for the production of  $\text{ClO}_4^-$  in all the UV mechanisms proposed.

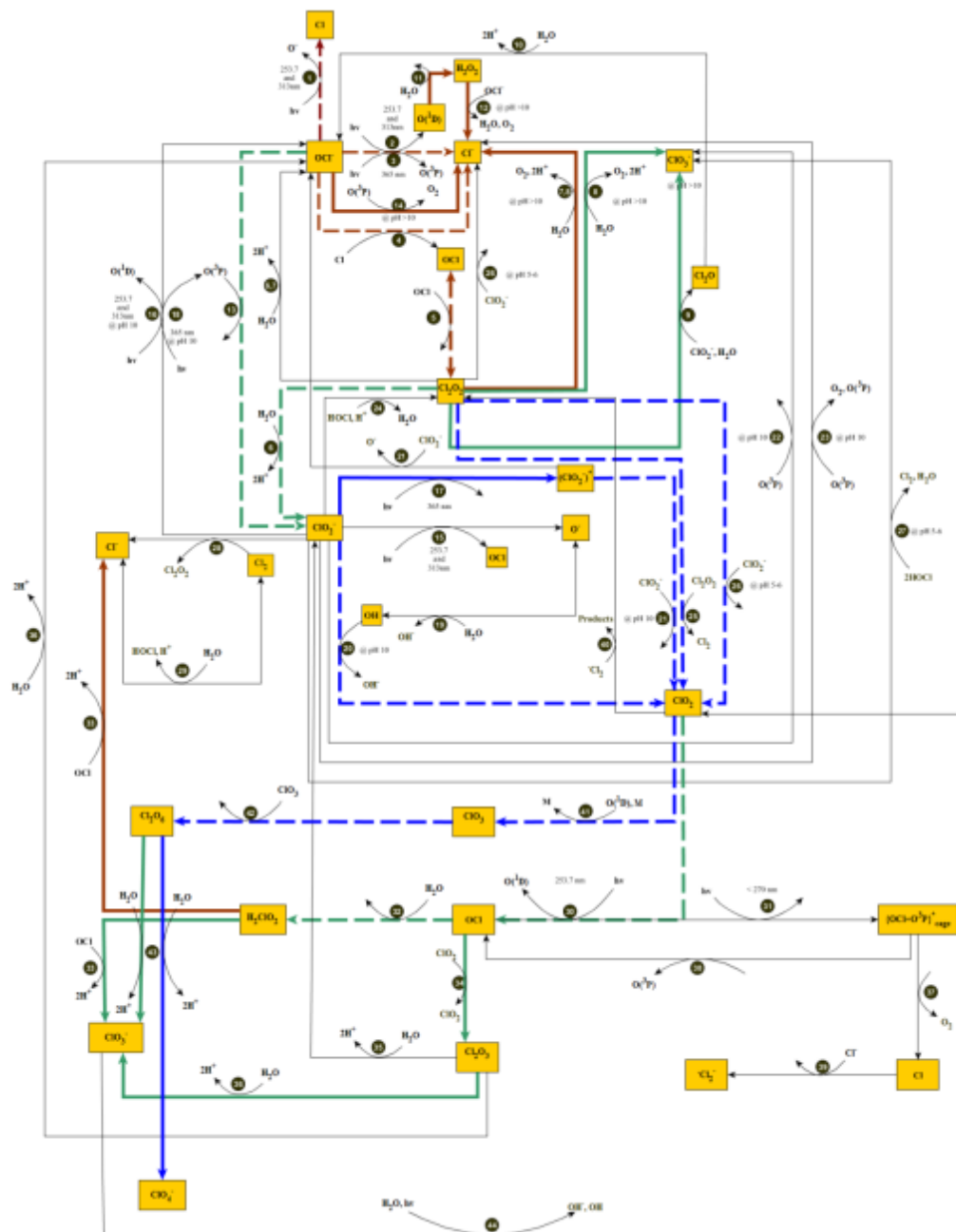


**Figure 2.5.24. UV oxidation of  $\text{OCl}^-$  (aq).** Note the thick red lines indicate the pathways leading to the formation of  $\text{Cl}^-$ , the thick aqua lines indicate the pathways leading to formation of  $\text{ClO}_3^-$ , and the thick blue lines indicate the pathways leading to the formation of  $\text{ClO}_4^-$ . 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-Chlorite***

Photodecomposition pathways of  $\text{ClO}_2^-$  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  $\text{ClO}_4^-$  formation from UV oxidation of  $\text{ClO}_2^-$  is not possible based on the number of O atoms from  $\text{O}_3$  as the  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  produced were below zero ( $\sim -2\text{‰}$ ), indicating that UV pathways are mass-dependent processes and that  $\text{O}_3$  was not a major source of O in  $\text{ClO}_4^-$ . The photolysis of  $\text{ClO}_2^-$  was observed at a wavelength of 350 nm and thus the  $\text{ClO}_4^-$  pathways proposed only include reactions occurring at longer wavelengths (365 nm) (Figure 2.5.25; Rxn's 17 and 18). All preferred UV  $\text{ClO}_4^-$  pathways lead to the formation of the  $\text{ClO}_2$  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  $\text{OCl}^-$  (Figure 2.5.24) to produce  $\text{ClO}_4^-$ .

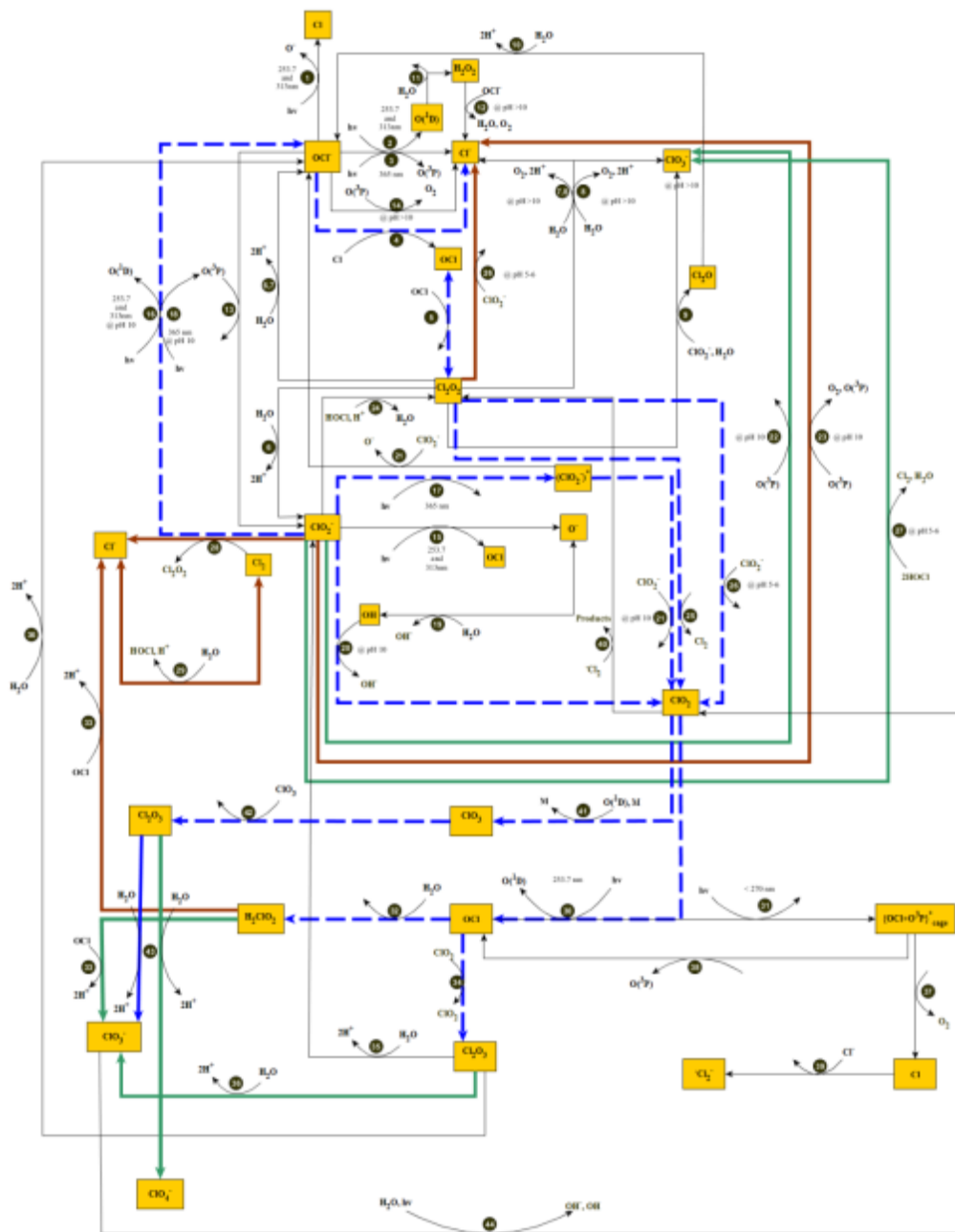


**Figure 2.5.25. UV oxidation of  $\text{ClO}_2^-$  (aq).** Note the thick red lines indicate the pathways leading to the formation of  $\text{Cl}$ , the thick aqua lines indicate the pathways leading to formation of  $\text{ClO}_3^-$ , and the thick blue lines indicate the pathways leading to the formation of  $\text{ClO}_4^-$ . 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***

$\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  formed from the photodecomposition of  $\text{ClO}_2$  (aq) (+ 0.9 ‰ and + 1.3 ‰) were higher than those of  $\text{ClO}_4^-$  from the  $\text{OCl}^-$  and  $\text{ClO}_2^-$  experiments indicating either an unusual mass-dependent process or possibly that  $\text{O}_3$  was generated by the UV reactions. These  $\Delta^{17}\text{O}$  values provide further evidence indicating that  $\text{ClO}_4^-$  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  $\text{O}_3$ . Based on what we know regarding UV oxidation of  $\text{OCl}^-$  and  $\text{ClO}_2^-$ , only one pathway was determined possible for the formation of  $\text{ClO}_4^-$  from UV oxidation of  $\text{ClO}_2$  (aq) (Figure 2.5.26; Rxn sequence: 30 and 41-43). This pathway requires the formation of the  $\text{ClO}_3$  radical (rxn 41) and the  $\text{Cl}_2\text{O}_6$  species (rxn 42).





#### 2.5.4.12 Implications of $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$

##### ***Expected Relationship Between $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ Values of $\text{ClO}_4^-$***

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

##### ***$\text{O}_3$ -Dry Chloride***

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

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

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

$$\begin{array}{l} \% \text{ O from } \text{H}_2\text{O} + \% \text{ O from } \text{O}_3 = \\ 1 \end{array} \quad (6)$$

$$(\% \text{ O from } \text{H}_2\text{O} \times \Delta^{17}\text{O of } \text{H}_2\text{O}) + (\% \text{ O from } \text{O}_3 \times \Delta^{17}\text{O of } \text{O}_3) = \Delta^{17}\text{O of } \text{ClO}_4^- \quad (7)$$

$$(\% \text{ O from } \text{H}_2\text{O} \times \delta^{18}\text{O of } \text{H}_2\text{O}) + (\% \text{ O from } \text{O}_3 \times \delta^{18}\text{O of } \text{O}_3) = \delta^{18}\text{O of } \text{ClO}_4^- \quad (8)$$

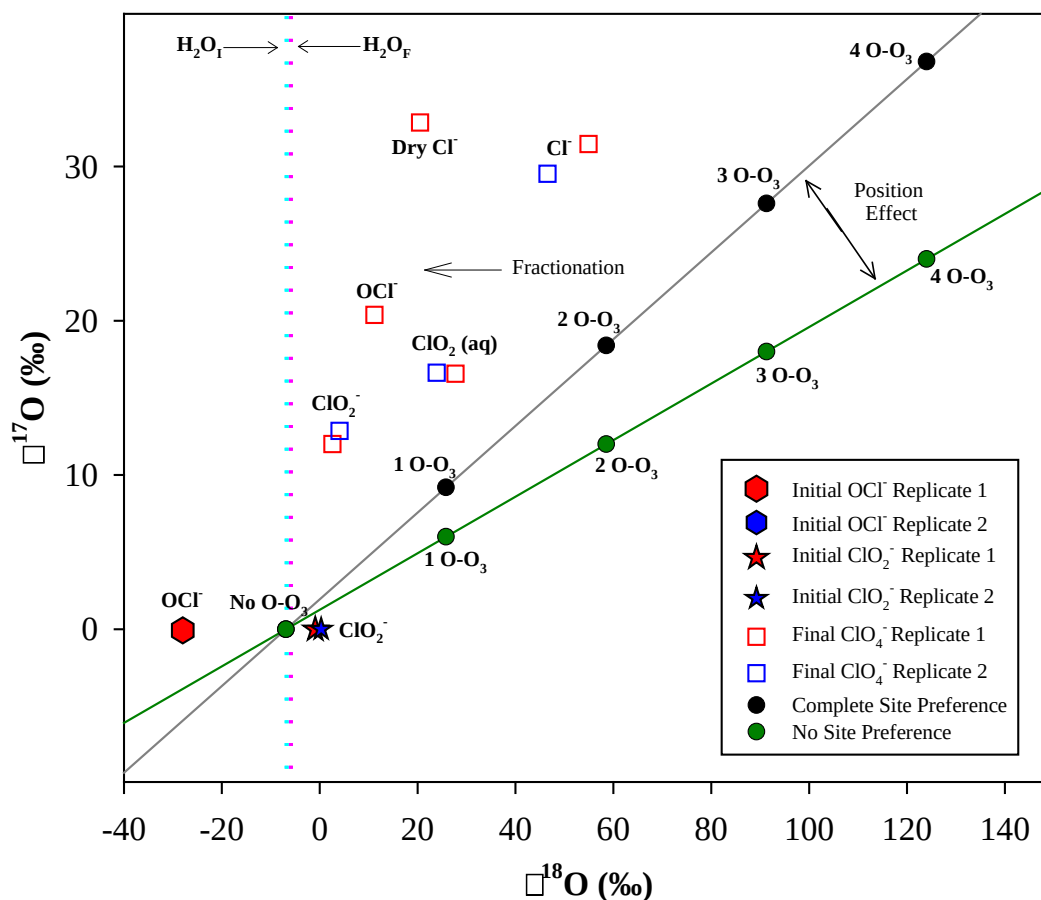


**Figure 2.5.27. Stable O isotopic compositions ( $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$ ) of reactant species and final  $\text{ClO}_4^-$  for  $\text{O}_3$  oxidized  $\text{ClO}_x$  solutions including expected  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  of  $\text{ClO}_4^-$  formed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

### ***$\text{O}_3$ -Chloride (aq)***

Similar to dry  $\text{Cl}^-$ ,  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  from the  $\text{Cl}^-$  (aq) experiments (+ 29 ‰ and + 32 ‰) also indicated that nearly all O atoms in  $\text{ClO}_4^-$  were from  $\text{O}_3$ , depending on the final  $\Delta^{17}\text{O}$  site preference value of  $\text{O}_3$ . The  $\text{ClO}_4^-$   $\delta^{18}\text{O}$  values were however higher (~ 25.5 – 34.5 ‰) than those of  $\text{ClO}_4^-$  from the dry  $\text{Cl}^-$  experiments but they were still lower (~ 69 – 78 ‰) than what would be expected (~ + 124 ‰) assuming no fractionation effects. Assuming that  $\text{O}_3$  had a final  $\Delta^{17}\text{O}$  site preference value of + 37 ‰, the maximum possible, it was determined using a  $\Delta^{17}\text{O}$

mass balance that between 13– 21% of O in  $\text{ClO}_4^-$  could come from  $\text{H}_2\text{O}$  and the other 79 – 87 % from  $\text{O}_3$  (Equations 6 and 7). It was determined through a  $\delta^{18}\text{O}$  mass balance that even assuming the maximum % of O that could come from  $\text{H}_2\text{O}$  (21%) would result in a  $\text{ClO}_4^-$  with a  $\delta^{18}\text{O}$  value of + 97 ‰, a value much greater than the  $\text{ClO}_4^-$   $\delta^{18}\text{O}$  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  $\delta^{18}\text{O}$  values of  $\text{ClO}_4^-$  formed from  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq), but that given the little that is known regarding O exchange between  $\text{ClO}_x$  species, that mass-dependent fractionation effects are the most likely explanation for the  $\delta^{18}\text{O}$  values of  $\text{ClO}_4^-$ .

### ***O<sub>3</sub>-Hypochlorite***

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

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

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

$$\% \text{ O from } \text{H}_2\text{O} + \% \text{ O from } \text{O}_3 + \% \text{ O from } \text{OCl}^- = 1 \quad (9)$$

$$(\% O \text{ from } H_2O \times \Delta^{17}O \text{ of } H_2O) + (\% O \text{ from } O_3 \times \Delta^{17}O \text{ of } O_3) + (\% O \text{ from } OCl^- \times \Delta^{17}O) = \Delta^{17}O \text{ of } ClO_4^- \quad (10)$$

$$(\% O \text{ from } H_2O \times \delta^{18}O \text{ of } H_2O) + (\% O \text{ from } O_3 \times \delta^{18}O \text{ of } O_3) + (\% O \text{ from } OCl^- \times \delta^{18}O) = \delta^{18}O \text{ of } ClO_4^- \quad (11)$$

### ***O<sub>3</sub>-Chlorite***

The O<sub>3</sub> oxidation of ClO<sub>2</sub><sup>-</sup> produced ClO<sub>4</sub><sup>-</sup> with the lowest Δ<sup>17</sup>O values (+ 12 ‰ and + 13 ‰) which indicated that two or less O in ClO<sub>4</sub><sup>-</sup> is from O<sub>3</sub>, depending on the final site preference value of O<sub>3</sub>. Assuming a Δ<sup>17</sup>O site preference value of + 37 ‰ for O<sub>3</sub>, the maximum possible and the measured Δ<sup>17</sup>O of H<sub>2</sub>O (0‰) and ClO<sub>2</sub><sup>-</sup> (+ 0.0 ‰) (assuming that the original O in the reactant ClO<sub>2</sub><sup>-</sup> species was transferred into the final ClO<sub>4</sub><sup>-</sup> structure), a mass balance determined that 15 – 17 % of O came from H<sub>2</sub>O, and 33–35 % of O came from O<sub>3</sub> (Equations 12 and 13). Out of the three O sources available (ClO<sub>2</sub><sup>-</sup>, H<sub>2</sub>O, and O<sub>3</sub>), bulk water has the lowest δ<sup>18</sup>O value (- 6.9 ‰). Assuming the maximum % of O that could come from water (17 %), based on the Δ<sup>17</sup>O mass balance, and the assumed 50 % of O that came from ClO<sub>2</sub><sup>-</sup>, we would expect a ClO<sub>4</sub><sup>-</sup> with a δ<sup>18</sup>O value near + 39 ‰, a value considerably greater than the measured ClO<sub>4</sub><sup>-</sup> δ<sup>18</sup>O values (+ 2 ‰ and + 4 ‰). The ClO<sub>4</sub><sup>-</sup> Δ<sup>17</sup>O values indicate that another O source other than O<sub>3</sub> is involved in the formation of ClO<sub>4</sub><sup>-</sup>, but given the mass balance results it appears that mixing alone cannot be responsible for the δ<sup>18</sup>O values observed. Given previous results for other oxidized species (Cl<sup>-</sup> and OCl<sup>-</sup>) the most likely explanation is that mass dependent fractionation and mixing of O from the original ClO<sub>2</sub><sup>-</sup> were both responsible for the δ<sup>18</sup>O values of ClO<sub>4</sub><sup>-</sup> from O<sub>3</sub> oxidation of ClO<sub>2</sub><sup>-</sup>.

$$\% O \text{ from } H_2O + \% O \text{ from } O_3 + \% O \text{ from } ClO_2^- = 1 \quad (12)$$

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

$$(\% O \text{ from } H_2O \times \delta^{18}O \text{ of } H_2O) + (\% O \text{ from } O_3 \times \delta^{18}O \text{ of } O_3) + (\% O \text{ from } ClO_2^- \times \delta^{18}O) = \delta^{18}O \text{ of } ClO_4^- \quad (14)$$

### ***O<sub>3</sub>-Chlorine Dioxide***

The  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  from  $\text{O}_3$  oxidation of  $\text{ClO}_2$  (aq) (+16 to +17 ‰) were similar to those of  $\text{ClO}_4^-$  from  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (+16, +17, and +20‰) but were somewhat higher than those of  $\text{ClO}_4^-$  from  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  (+ 12 ‰ and + 13 ‰). However, the  $\delta^{18}\text{O}$  values of  $\text{ClO}_4^-$  formed in the  $\text{ClO}_2$  (aq) experiments (+ 23 ‰ to + 28 ‰) were higher than the  $\delta^{18}\text{O}$  values of  $\text{ClO}_4^-$  from both  $\text{O}_3$  oxidation of  $\text{OCl}^-$  and  $\text{ClO}_2^-$ : (+ 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  $\text{ClO}_2$  (aq) experiments as  $^{18}\text{O}$  isotopes for the starting  $\text{ClO}_2$  (aq) were not measured.

### ***UV-Hypochlorite***

Photodecomposition of  $\text{OCl}^-$  produced a  $\text{ClO}_4^-$  with near zero  $\Delta^{17}\text{O}$  values (-0.0 ‰ to + 0.5 ‰) and the lowest  $\delta^{18}\text{O}$  values (- 19 ‰ to - 22 ‰) out of all the UV experiments.  $\text{ClO}_4^-$  pathways proposed for UV oxidation of  $\text{OCl}^-$  (Figure 2.5.24) indicate at least five O sources are available for  $\text{ClO}_4^-$  formation: initial  $\text{OCl}^-$ ,  $\text{O}(^3\text{P})$ , OH,  $\text{OCl}$ , and  $\text{H}_2\text{O}$ . Initial  $\text{OCl}^-$  and  $\text{H}_2\text{O}$  had low  $\delta^{18}\text{O}$  values (-28 ‰ and -6.9‰, respectively), but the  $\Delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  values of  $\text{O}(^3\text{P})$ , OH, and  $\text{OCl}$  are not available, thus a proper evaluation of the percentage of O in  $\text{ClO}_4^-$  from the different O sources cannot be made.

### ***UV-Chlorite***

The photolysis of  $\text{ClO}_2^-$  led to the production of  $\text{ClO}_4^-$  with  $\Delta^{17}\text{O}$  values relatively close to zero (- 1 ‰ to -2 ‰) but with high  $\delta^{18}\text{O}$  values (+ 42 ‰ to + 46 ‰). Only three O sources were identified in the proposed  $\text{ClO}_4^-$  mechanisms (Figure 2.5.25) as available for  $\text{ClO}_4^-$  formation from UV exposure to  $\text{ClO}_2^-$ : initial  $\text{ClO}_2^-$ , the  $\text{OCl}$  radical, and  $\text{H}_2\text{O}$ . Because there is lack of information related to the  $\delta^{18}\text{O}$  values of the  $\text{OCl}$  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  $\delta^{18}\text{O}$  values of initial  $\text{ClO}_2^-$  (- 2.5 ‰) and  $\text{H}_2\text{O}$  (- 6.9 ‰) were both below zero it can be deduced that for the  $\text{ClO}_4^-$  formed to be a result of mixing of the O sources, the  $\delta^{18}\text{O}$  value of the  $\text{OCl}$  radical would have to be higher than the  $\delta^{18}\text{O}$  values of the  $\text{ClO}_4^-$  reagent or that there was substantial O isotope fractionation.

### ***UV-Chlorine Dioxide***

The photodecomposition of  $\text{ClO}_2$  (aq) resulted in  $\text{ClO}_4^-$  with  $\Delta^{17}\text{O}$  values near zero (+ 0.9 ‰ and +1.3‰) and with  $\delta^{18}\text{O}$  values (+ 9 ‰ to +13 ‰) higher than those of the  $\text{OCl}^-$  experiments (- 19‰ to -22‰) but lower than those of the  $\text{ClO}_2^-$  experiments (+ 42 ‰ to + 46 ‰). Two initial O sources were available in the UV  $\text{ClO}_2$  (aq) experiments: initial  $\text{ClO}_2$  (aq) and  $\text{H}_2\text{O}$ . The  $\delta^{18}\text{O}$  values for initial  $\text{ClO}_2$  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  $\delta^{18}\text{O}$  values of initial  $\text{ClO}_2$  (aq)

would have to be higher than the  $\delta^{18}\text{O}$  values of  $\text{H}_2\text{O}$  and  $\text{ClO}_4^-$  for mixing to be responsible for the  $\text{ClO}_4^-$   $\delta^{18}\text{O}$  values.

#### 2.5.4.13 Fractionation

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

Kinetic isotope fractionation would result in a large fractionation effect, depending on the pathway leading to  $\text{ClO}_4^-$ , 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  $\text{ClO}_4^-$  formation mechanisms (Figure 2.5.16–2.5.23) are known and reaction sequences are speculative, thus it is not possible to determine if  $\delta^{18}\text{O}$  values of  $\text{ClO}_4^-$  resulted from kinetic fractionation and/or equilibrium isotope exchange reactions.

#### ***Implications of $\delta^{37}\text{Cl}$***

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

The high  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  produced in the UV and  $\text{O}_3$  experiments may have been influenced by equilibrium or kinetic fractionations between different aqueous  $\text{ClO}_x$  species. Mixing calculations are not possible due to the lack of  $^{37}\text{Cl}$  isotope data for all possible Cl sources in both UV and  $\text{O}_3$  experiments, but it is hypothesized that because  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  produced in all aqueous  $\text{O}_3$  and UV experiments were relatively high, that the  $\delta^{37}\text{Cl}$  values of the Cl sources would need to be relatively high as well for mixing to have occurred while in the  $\text{O}_3$



dry  $\text{Cl}^-$  experiment, the  $\delta^{37}\text{Cl}$  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  $\delta^{37}\text{Cl}$  values.

Kinetic Cl isotope fractionation factors related to photodecomposition or  $\text{O}_3$  oxidation of  $\text{Cl}^-$ ,  $\text{OCl}^-$ ,  $\text{ClO}_2^-$ , and  $\text{ClO}_2$  have not been experimentally determined. Schauble et al.<sup>49</sup> determined that under equilibrium conditions at 298 K,  $\delta^{37}\text{Cl}$  values of coexisting  $\text{ClO}_x$  species were possibly correlated with Cl oxidation state:  $\text{Cl}^{-1}$ ,  $\text{Cl}^0$ ,  $\text{Cl}^{+1}$ ,  $\text{Cl}^{+2}$ ,  $\text{Cl}^{+3}$ ,  $\text{Cl}^{+4}$ , and  $\text{Cl}^{+7}$  can concentrate  $^{37}\text{Cl}$  approximately by +2.5 ‰ to +6 ‰, +7 ‰, +7 ‰ to +9 ‰, +10 ‰, +27 ‰, and +75 ‰, respectively. Any reversible reaction in the proposed  $\text{ClO}_4^-$  mechanisms for either UV or  $\text{O}_3$  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  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  produced in the aqueous UV (+17 ‰ to +23 ‰) and  $\text{O}_3$  (+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  $\text{ClO}_4^-$ . One example of this is reaction 4 (Table 2.5.4) from the  $\text{O}_3$  proposed mechanisms, a reaction for which no forward or reverse reaction rates are known. If  $\text{ClO}_2$  was converted to  $\text{ClO}_3$  with relatively little kinetic isotope fractionation while also being consumed by another reaction with a larger kinetic fractionation, the  $\delta^{37}\text{Cl}$  of  $\text{ClO}_3$  could increase rapidly and thus produce  $\text{ClO}_4^-$  with high  $\delta^{37}\text{Cl}$  values. This is purely hypothesized but illustrates that high  $\delta^{37}\text{Cl}$   $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{Cl}_2\text{O}_6$  species being hydrolyzed to produce  $\text{ClO}_4^-$  and  $\text{ClO}_3^-$ . In this reaction (Rxn 43) the  $^{37}\text{Cl}$  isotope may be located in the  $\text{Cl}_2\text{O}_6$  species in such a way that when hydrolyzed, always ends up in  $\text{ClO}_4^-$ . Once formed  $\text{ClO}_4^-$  is not known to further react thus we do not know if this phenomenon is possible outside of equilibrium.

Given the high  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  from the aqueous UV and  $\text{O}_3$  experiments and the low  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  from the  $\text{O}_3$  dry  $\text{Cl}^-$  experiments, it appears that heterogeneous surfaces can undergo different isotope fractionation processes than do homogenous surfaces. The low  $\delta^{37}\text{Cl}$

value of  $\text{ClO}_4^-$  produced from  $\text{Cl}^-$  in the dry UV experiments implies kinetic fractionations were more important than equilibrium effects in the dry system.

### ***Relation to Natural $\text{ClO}_4^-$***

All  $\text{ClO}_4^-$  generated in the UV and  $\text{O}_3$  aqueous experiments was as heavy as or heavier in  $\delta^{37}\text{Cl}$  than natural  $\text{ClO}_4^-$  (Figure 2.5.28). Our  $\text{ClO}_4^-$   $\delta^{37}\text{Cl}$  isotope data further suggest that aqueous production mechanisms produce  $\text{ClO}_4^-$  that is enriched in  $^{37}\text{Cl}$  whereas dry production mechanisms produce  $\text{ClO}_4^-$  depleted in  $^{37}\text{Cl}$ . Given this information, if aqueous reactions were solely responsible for  $\text{ClO}_4^-$  production in the atmosphere and for natural  $\text{ClO}_4^-$ , then it would mean that precursor species, prior to  $\text{ClO}_4^-$  formation, would need to have considerably lower  $\delta^{37}\text{Cl}$  values than Atacama  $\text{ClO}_4^-$ , which has the lowest reported  $\delta^{37}\text{Cl}$  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  $\delta^{37}\text{Cl}$  than Atacama  $\text{ClO}_4^-$ , it is difficult to conceive an atmospheric production process that is purely dependent upon aqueous reactions. Given the lower  $\delta^{37}\text{Cl}$  values of  $\text{ClO}_4^-$  from the  $\text{O}_3$  dry  $\text{Cl}^-$  experiments and those of natural  $\text{ClO}_4^-$ , it is more likely that natural  $\text{ClO}_4^-$  is a result of dry (heterogeneous) atmospheric reactions (Figure 2.5.28). Dry production mechanisms could potentially start with a precursor species with high  $\delta^{37}\text{Cl}$  values, as high as those reported for species found on Earth, and would end up producing a  $\text{ClO}_4^-$  with lower  $\delta^{37}\text{Cl}$  values, a finding that is more consistent with the reported  $\delta^{37}\text{Cl}$  values of natural  $\text{ClO}_4^-$ .

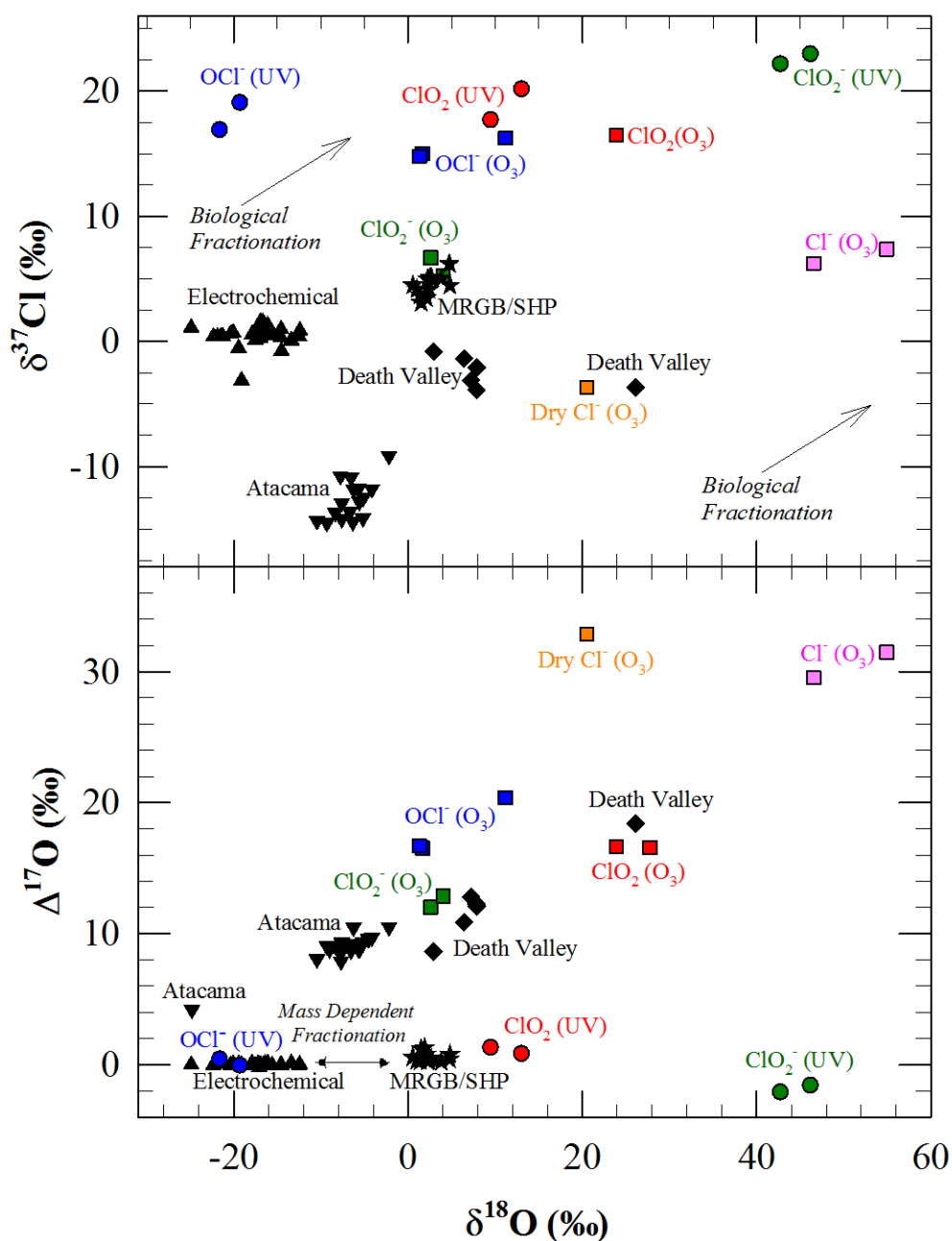


**Figure 2.5.28. Summary of isotope data for  $\text{ClO}_4^-$  produced in the  $\text{O}_3$  (square symbols) and UV (circle symbols) generation experiments.** Species labels next to the  $\text{ClO}_4^-$  symbols represent the precursor species from which  $\text{ClO}_4^-$  was produced. Generation data is displayed with previously published isotope data for  $\text{ClO}_4^-$  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  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  produced in the  $\text{O}_3$  experiments fell along a diagonal, positive line indicating different mixing reactions between  $\text{O}_3$  and other O sources along with possible fractionation and/or isotope exchange (Figure 2.5.28). Our  $\text{O}_3$  experiment  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  isotope data is consistent with the trend observed between  $\delta^{18}\text{O}$  and  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  found in Death Valley and the Atacama caliche, indicating that  $\text{ClO}_4^-$  in these regions consistent with  $\text{O}_3$  production mechanisms (Figure 2.5.28).  $\text{ClO}_4^-$  from the UV experiments did not have positive  $\Delta^{17}\text{O}$  values but the  $\delta^{18}\text{O}$  values largely bracketed the  $\delta^{18}\text{O}$  values observed for MRGB/SHP  $\text{ClO}_4^-$ , indicating that indigenous  $\text{ClO}_4^-$  in this region could be consistent with UV production mechanisms (Figure 2.5.28). Coincidentally, natural  $\text{ClO}_4^-$  that is consistent with  $\text{O}_3$  production mechanisms (Atacama and Death Valley) had negative  $\delta^{37}\text{Cl}$  values while natural  $\text{ClO}_4^-$  that is consistent with UV mechanisms (MRGB/SHP) had positive  $\delta^{37}\text{Cl}$  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  $\text{ClO}_4^-$  found on Earth.

#### ***Uncertainty Related to $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ 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  $\delta^{18}\text{O}$  and (2) the  $\text{O}_2$  dual-inlet isotope-ratio mass spectrometry (O2-DIIRMS) method which is used to measure  $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  (Bohlke et al., 2016). The average difference between  $\delta^{18}\text{O}$  values from each method was  $1.55 \pm 1.29$  ‰. Only the  $\delta^{18}\text{O}$  values obtained from the O2-DIIRMS method were reported and used for  $\Delta^{17}\text{O}$  calculations in this study as  $\delta^{18}\text{O}$  values from the CO-CFIRMS method were not available for all species.

$\Delta^{17}\text{O}$  values are also considered an effect of different equations and different coefficients/exponents ( $\lambda$ ) (Table 2.5.5). Many definitions of  $\Delta^{17}\text{O}$  have been formulated, with the coefficient ( $\lambda$ ) ranging from 0.50 – 0.53 depending on the type of conditions and processes being observed, and can result in different  $\Delta^{17}\text{O}$  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  $\lambda$  value of 0.525. Because  $\Delta^{17}\text{O}$  variations in values of  $\text{ClO}_4^-$  from the UV experiments are small, we must consider the variability in  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  had we used other definitions. The  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  calculated using different definitions of  $\Delta^{17}\text{O}$  and  $\lambda$  range of 0.50 – 0.53 are reported in Table 2.5.5 and indicate that the photodecomposition of  $\text{OCl}^-$  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  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  from the UV  $\text{ClO}_2^-$  and  $\text{ClO}_2$  (aq) experiments are truly negative and positive, respectively, indicating some mass-independent fractionation (Table 2.5.5).

**Table 2.5.5. Calculated  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  produced in the UV experiments using the coefficient ( $\lambda$ ) range of 0.50 – 0.53 in the various definitions of  $\Delta^{17}\text{O}$ .**

| Definition/Equation                                                                                                  | Reference                                  | Possible Range in $\Delta^{17}\text{O}$ Values of $\text{ClO}_4^-$ in UV Experiments (‰) |                   |                   |                   |                   |                   |
|----------------------------------------------------------------------------------------------------------------------|--------------------------------------------|------------------------------------------------------------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                                                                                                      |                                            | $\text{OCI}^-$                                                                           |                   | $\text{ClO}_2^-$  |                   | $\text{ClO}_2$    |                   |
|                                                                                                                      |                                            | Rep 1                                                                                    | Rep 2             | Rep 1             | Rep 2             | Rep 1             | Rep 2             |
| <sup>b</sup> $\Delta^{17}\text{O} = k = \frac{(1 + \delta^{17}\text{O})}{(1 + \delta^{18}\text{O})^k} - 1$           | Miller, 2002                               | (-0.52) – (+0.07)                                                                        | (-0.10) – (+0.56) | (-1.02) – (-2.28) | (-0.43) – (-1.78) | (+1.20) – (+0.81) | (+1.57) – (+1.28) |
| <sup>a</sup> $\Delta^{17}\text{O} = \delta^{17}\text{O} - (\lambda \times \delta^{18}\text{O})$                      | Cliff and Thiemens, 1997<br>Thiemens, 1999 | (-0.56) – (+0.02)                                                                        | (-0.15) – (+0.49) | (-1.27) – (-2.55) | (-0.70) – (-2.08) | (+1.19) – (+0.79) | (+1.56) – (+1.28) |
| <sup>a</sup> $\Delta^{17}\text{O} = (1 + \delta^{17}\text{O}) - (1 + \delta^{18}\text{O})^\lambda$                   | Farquhar et al., 1999                      | (-0.51) – (+0.07)                                                                        | (-0.10) – (+0.55) | (-1.04) – (-2.32) | (-0.44) – (-1.82) | (+1.21) – (+0.82) | (+1.58) – (+1.29) |
| <sup>b</sup> $^{17}\Delta = \ln(k + 1) = \ln(1 + \delta^{17}\text{O}) - \lambda \times \ln(1 + \delta^{18}\text{O})$ | Luz and Barkan, 2005                       | (-0.52) – (+0.07)                                                                        | (-0.10) – (+0.56) | (-1.02) – (-2.28) | (-0.43) – (-1.78) | (+1.20) – (+0.81) | (+1.57) – (+1.28) |

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

<sup>b</sup> This definition characterizes a specific mass-dependent fractionation (MDF) line, where  $^{17}\Delta$  is the ordinate intercept (Miller, 2002; Luz and Barkan, 2005; Assonov and Brenninkmeijer, 2005)

### 2.5.5 Conclusions

This study presents proposed reaction mechanisms for the formation of  $\text{ClO}_4^-$ , with distinct isotopic compositions ( $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ , and  $\Delta^{17}\text{O}$ ), via  $\text{O}_3$  oxidation and UV photolysis. While no new UV production pathways were introduced, we were able to partially constrain  $\text{O}_3$  formation pathways based on the number of O atoms in  $\text{ClO}_4^-$  coming from  $\text{O}_3$ , as indicated by  $\Delta^{17}\text{O}$  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  $\text{ClO}_4^-$  formed from oxidation (UV and  $\text{O}_3$ ) of various  $\text{ClO}_x$  species is a result of mixing between  $\text{ClO}_4^-$  formed from branching pathways along with some fractionation, with UV  $\text{ClO}_4^-$  isotope data suggesting mass-dependent fractionation and  $\text{O}_3$   $\text{ClO}_4^-$  isotope data being more consistent with mass-independent variation. There is also evidence to suggest that oxidation of  $\text{ClO}_x$  species on a heterogeneous (dry) surface has different fractionation effects than oxidation of fully aqueous  $\text{ClO}_x$  species.

From the  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  formed in the  $\text{O}_3$  experiments we were able to show a large  $\text{O}_3$  position effect, indicating that the heavy isotope is preferentially located in the terminal O atoms in  $\text{O}_3$  and that the terminal O atoms were preferentially incorporated into  $\text{ClO}_x$  compounds.  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  were approximately related to the number of O atoms added from  $\text{O}_3$  and indicated a stepwise reduction in the contribution of O atoms from  $\text{O}_3$  into  $\text{ClO}_4^-$  based on increasing oxidation state of Cl in precursor species. In addition, the  $\Delta^{17}\text{O}$  data for the  $\text{O}_3$  experiments indicated large quantities of  $\text{ClO}_3^-$  were produced by a process that was not involved in production of  $\text{ClO}_4^-$ , meaning that  $\text{ClO}_3^-$  was not the major precursor for  $\text{ClO}_4^-$  in the  $\text{O}_3$  experiments. The  $\delta^{37}\text{Cl}$  of  $\text{ClO}_4^-$  was generally higher than precursor  $\delta^{37}\text{Cl}$  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  $\text{ClO}_4^-$  that incorporate much of the reported variation in nature. The low  $\delta^{37}\text{Cl}$  in Atacama  $\text{ClO}_4^-$  was not reproduced, but all other variations were. All other isotopic data suggests that  $\text{ClO}_4^-$  indigenous to Atacama and Death Valley and  $\text{ClO}_4^-$  from MRGB/SHP are consistent with an  $\text{O}_3$  and a UV related  $\text{ClO}_4^-$  mechanism, respectively. Unfortunately we cannot determine which precursor species were involved in the formation of  $\text{ClO}_4^-$  in each of these regions. Our experiments showed that final  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  formed through mechanisms in our atmosphere would be the same. The reaction rates and intermediate species proposed to be involved in  $\text{ClO}_4^-$  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  $\text{Cl}^-$ , on a heterogeneous surface or gas phase would provide additional information to resolve ambiguities such as  $\delta^{37}\text{Cl}$  of Atacama  $\text{ClO}_4^-$ .

## 2.6 Evaluation of Bacterial Production of Perchlorate

### 2.6.1 Background

One potential production mechanism for natural  $\text{ClO}_4^-$  that has not been previously evaluated is microbiological generation. Nitrifying bacteria generate nitrate through the oxidation of  $\text{NH}_4$  (e.g., *Nitrosomonas* spp.) and  $\text{NO}_2^-$  (e.g., *Nitrobacter* spp.) (Paerl, 1997). During this well-studied process,  $\text{NH}_4$  serves as a microbial electron donor and oxygen as an electron acceptor (Capone, 1997). There are also anaerobic oxidation processes by which  $\text{NO}_3^-$  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  $\text{Cl}^-$  to hypochlorous acid ( $\text{HClO}$ ) (Griffin, 1991; Vilter, 1995). This could represent an initial biological step in the production of natural  $\text{ClO}_4^-$  in some environments (possibly followed by additional photochemical or biological reactions). We should also note that the oxygen isotope ratios reported for  $\text{ClO}_4^-$  in West Texas are similar to those of biologically generated  $\text{NO}_3^-$ , which incorporates O from  $\text{H}_2\text{O}$  and  $\text{O}_2$  during microbial oxidation of  $\text{NH}_4$  (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  $\text{ClO}_4^-$ . During this task, we evaluated whether microbial generation of  $\text{ClO}_4^-$  (as well as  $\text{HClO}$ ,  $\text{ClO}_2^-$ , and  $\text{ClO}_3^-$ ) 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  $\text{ClO}_4^-$  during the oxidation of chloride ( $\text{Cl}^-$ ), both in the presence and absence of ultraviolet (UV) light from natural sunlight. Haloperoxidases combine hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) with halides (e.g.,  $\text{Cl}^-$ ,  $\text{I}^-$ ,  $\text{Br}^-$ ) to form corresponding hypohalous acids (e.g.,  $\text{HClO}$  from  $\text{Cl}^-$ ) (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  $\text{ClO}_4^-$  during the oxidation of  $\text{Cl}^-$ , both in the presence and absence of UV light. Follow-on studies were conducted to better refine conditions of  $\text{ClO}_4^-$  generation. To our knowledge the potential for these enzymes to produce  $\text{ClO}_4^-$ , either as a minor side reaction, or through the subsequent interaction of the biologically-generated  $\text{HClO}$  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  $\mu$ l 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  $\text{ClO}_4^-$  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  $\text{H}_2\text{O}_2$ . 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  $\text{ClO}_4^-$  in the test samples. The individual components (buffer,  $\text{H}_2\text{O}_2$ , and diluted enzyme) were analyzed separately. Furthermore, mixtures of the components (buffer + enzyme and buffer +  $\text{H}_2\text{O}_2$ ) 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  $\text{H}_2\text{O}_2$  concentration on the enzymatic production of  $\text{ClO}_4^-$  were assessed. Results are detailed below.

##### ***Analysis***

At the conclusion of each experiment, samples were analyzed for  $\text{ClO}_4^-$  in the laboratory of Andrew Jackson at Texas Tech University using IC/MS/MS as described previously in this report. The detection limit for  $\text{ClO}_4^-$  by this technique is ~ 0.05  $\mu\text{g/L}$ .

#### 2.6.2.2 Results and Discussion

All of the components that comprise the complete reaction mixture were analyzed for the presence of  $\text{ClO}_4^-$  as separate entities (buffer,  $\text{H}_2\text{O}_2$ , and the enzyme mix diluted in water). The  $\text{H}_2\text{O}_2$  had the highest levels of  $\text{ClO}_4^-$  (1.88  $\mu\text{g/L}$ ), while the diluted enzyme and buffer had much

lower concentrations (0.33 and 0.08  $\mu\text{g/L}$  respectively). The contribution of  $\text{ClO}_4^-$  from the  $\text{H}_2\text{O}_2$  in the reaction mixture is  $\sim 1000\times$  less than the reported concentration due to dilution (i.e., only 0.002  $\mu\text{g/L}$ ). Based on the initial results, the background  $\text{ClO}_4^-$  concentration in the typical reaction mixture should equal  $\sim 0.4 \mu\text{g/L}$  or less.

In the initial study with vanadium chloroperoxidase (VCP), production of  $\text{ClO}_4^-$  was not apparent when samples were incubated in the dark, but there was an increase in  $\text{ClO}_4^-$  in those incubated for  $\sim 8$  h in sunlight (Table 2.6.1). The mean  $\text{ClO}_4^-$  concentration in the samples exposed to UV light (Full rxn – light in Table 2.6.1) was 0.86  $\mu\text{g/L}$  whereas that in the samples remaining in the dark was 0.32  $\mu\text{g/L}$ , 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  $\text{ClO}_4^-$  was evaluated in the presence and absence of UV light (Table 2.6.2). These data again showed significant variability among treatments, with mean  $\text{ClO}_4^-$  concentrations ranging from 0.3 to 0.8  $\mu\text{g/L}$ . The enzyme reaction performed in sunlight yielded higher  $\text{ClO}_4^-$  than that performed in the dark ( $0.61 \pm 0.09 \mu\text{g/L}$  vs  $0.27 \pm 0.08 \mu\text{g/L}$ , respectively), but both values were lower than that with buffer and  $\text{H}_2\text{O}_2$  alone (i.e., no enzyme) at  $0.76 \pm 0.46 \mu\text{g/L}$ . In a subsequent study, the influence of  $\text{H}_2\text{O}_2$  concentration on  $\text{ClO}_4^-$  production was evaluated. All reactions were exposed to sunlight for  $\sim 8$  h. As shown in Table 2.6.3, the  $\text{ClO}_4^-$  concentrations ranging from  $\sim 1$  to 4  $\mu\text{g/L}$  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 H<sub>2</sub>O<sub>2</sub> and Cl<sup>-</sup> with vanadium chloroperoxidase (VCP).**

| Sample                                         | PC [ppb] | Range | N= |
|------------------------------------------------|----------|-------|----|
| Buffer + enzyme - dark                         | 0.31     | 0.19  | 2  |
| Buffer + enzyme - light                        | 0.30     | 0.18  | 2  |
| Buffer + H <sub>2</sub> O <sub>2</sub> - dark  | 0.35     | 0.25  | 2  |
| Buffer + H <sub>2</sub> O <sub>2</sub> - light | 0.34     | 0.23  | 2  |
| Full rxn - dark                                | 0.32     | 0     | 2  |
| Full rxn - light                               | 0.86     | 0.6   | 2  |

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

| Sample                                 | ClO <sub>4</sub> <sup>-</sup> [µg/L] | Range | N |
|----------------------------------------|--------------------------------------|-------|---|
| Buffer + Enzyme                        | 0.42                                 | 0.26  | 3 |
| Buffer + H <sub>2</sub> O <sub>2</sub> | 0.76                                 | 0.46  | 3 |
| Full rxn - dark                        | 0.27                                 | 0.08  | 3 |
| Full rxn - light                       | 0.61                                 | 0.09  | 3 |

**Table 2.6.3. Evaluation of the influence of H<sub>2</sub>O<sub>2</sub> concentration on perchlorate production by fungal iron chloroperoxidase.**

| Sample                                | ClO <sub>4</sub> <sup>-</sup> [µg/L] | Range | N |
|---------------------------------------|--------------------------------------|-------|---|
| 0.5 mM H <sub>2</sub> O <sub>2</sub>  | 1.02                                 | 0.41  | 2 |
| 2.5mM H <sub>2</sub> O <sub>2</sub>   | 2.2                                  | 1.2   | 2 |
| 12.5 mM H <sub>2</sub> O <sub>2</sub> | 3.96                                 | 2.9   | 2 |
| 62.5 mM H <sub>2</sub> O <sub>2</sub> | 2.85                                 | 3.3   | 2 |

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  $\text{ClO}_4^-$  in the presence/absence of sunlight and  $\text{H}_2\text{O}_2$ . The data showed high variability, but provided some indication that  $\text{ClO}_4^-$  production was possible in samples incubated in sunlight with chloroperoxidase enzyme and  $\text{H}_2\text{O}_2$ . 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  $\text{HClO}$  (or  $\text{ClO}^\cdot$ ), which can subsequently be photochemically converted to  $\text{ClO}_4^-$  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,  $\text{H}_2\text{O}_2$  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  $\text{ClO}_4^-$  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 H<sub>2</sub>O<sub>2</sub>, 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 H<sub>2</sub>O<sub>2</sub>, 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 ClO<sub>4</sub><sup>-</sup> 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 100mM 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 H<sub>2</sub>O<sub>2</sub> (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 ( $A_{278}$ ) 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 ClO<sub>4</sub><sup>-</sup> formation occurred. The background ClO<sub>4</sub><sup>-</sup> 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  $A_{278}$  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 ClO<sub>4</sub><sup>-</sup> 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  $\text{ClO}_4^-$  was formed when assays were exposed either to artificial UV light or to natural sunlight. The reason for this failure to produce  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ . The chloroperoxidase experiments were concluded at this point. It is possible that low levels of  $\text{ClO}_4^-$  are generated via this mechanism in nature, but unlikely that this is a significant source of natural  $\text{ClO}_4^-$  in the environment.

**Table 2.6.4. Perchlorate levels ( $\mu\text{g/L}$ ) in UV light experiments.**

| Buffer Used                 | pH | Perchlorate ( $\mu\text{g/L}$ ) |                    |                   |                     |                         |                   |                   |
|-----------------------------|----|---------------------------------|--------------------|-------------------|---------------------|-------------------------|-------------------|-------------------|
|                             |    | $\text{ClO}_4^-$ in Buffer      | Control Treatments |                   |                     | Experimental Treatments |                   |                   |
|                             |    |                                 | No UV control      | No enzyme control | No peroxide control | Fumago CPO (Sigma)      | Fumago CPO (BR)   | SBP (BR)          |
| 100 mM $\text{PO}_4$ buffer | 5  | $0.18 \pm 0.16^{* \wedge}$      | ND                 | ND                | ND                  | $0.23 \pm 0.28^*$       | $0.14 \pm 0.12^*$ | <0.26             |
| 100 mM $\text{PO}_4$ buffer | 7  | $0.48 \pm 0.59^{* \wedge}$      | ND                 | ND                | ND                  | $0.35 \pm 0.49^*$       | $0.55 \pm 0.61^*$ | $0.19 \pm 0.21^*$ |
| 10 mM $\text{PO}_4$ buffer  | 3  | $3.44 \pm 0.03^{\dagger}$       | ND                 | ND                | ND                  | $3.78 \pm 0.04$         | ND                | $3.75 \pm 0.11$   |
| 10 mM $\text{PO}_4$ buffer  | 5  | $0.15 \pm 0.15^*$               | $0.28 \pm 0.01$    | $0.41 \pm 0.18$   | $0.36 \pm 0.13$     | $0.70 \pm 0.60$         | ND                | $0.42 \pm 0.09$   |
| 10 mM $\text{PO}_4$ buffer  | 7  | <0.25                           | ND                 | ND                | ND                  | $0.53 \pm 0.81^*$       | ND                | $0.46 \pm 0.18$   |

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

**Table 2.6.5. Perchlorate levels ( $\mu\text{g/L}$ ) in natural sunlight experiments.**

|                                | 2 hours         |                 | 4 hours         |                 | sample temperature ( $^{\circ}\text{C}$ ) |
|--------------------------------|-----------------|-----------------|-----------------|-----------------|-------------------------------------------|
|                                | Light           | Dark            | Light           | Dark            |                                           |
| samples on ice                 | $0.37 \pm 0.03$ | $0.35 \pm 0.04$ | $0.39 \pm 0.07$ | $0.39 \pm 0.02$ | $17 \pm 5$                                |
| samples at ambient temperature | $0.45 \pm 0.08$ | $0.66 \pm 0.15$ | $0.60 \pm 0.05$ | $0.57 \pm 0.04$ | $39 \pm 9$                                |

Perchlorate values in  $\mu\text{g/L}$   
 Perchlorate values are the average of duplicate values +/- one standard deviation

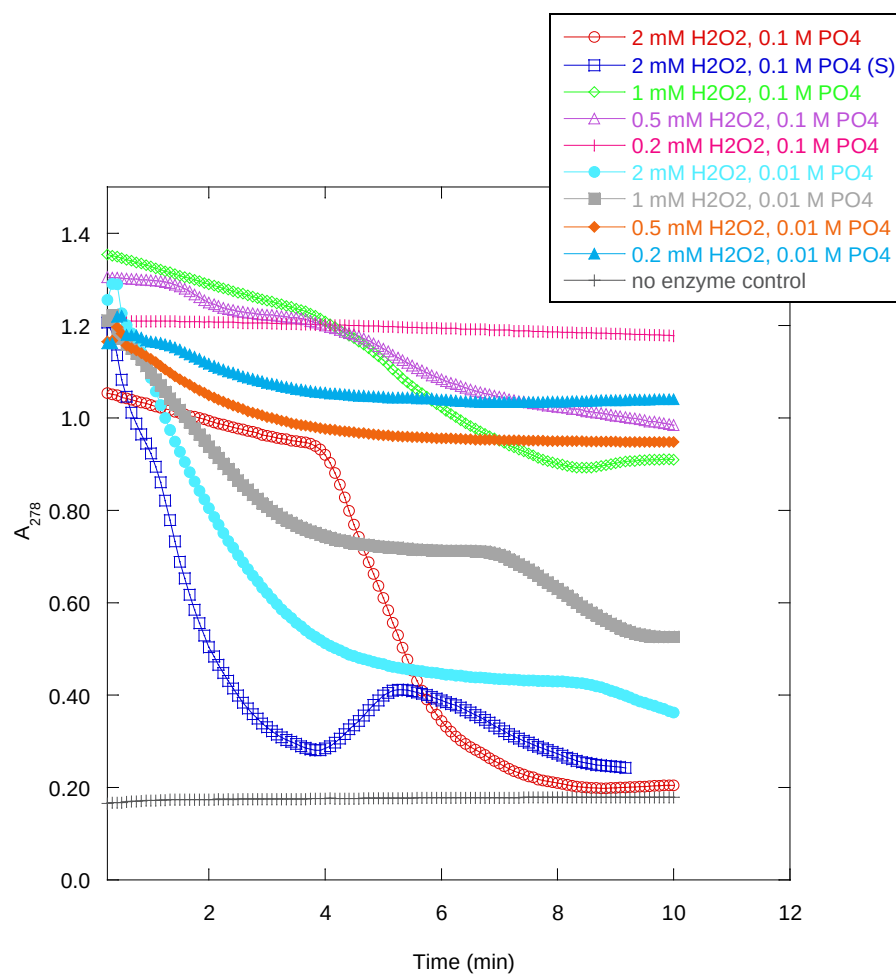


**Figure 2.6.1. Results of MCD assays.** Decreases in  $A_{278}$  indicate the loss of monochlorodimedone as it is converted to dichlorodimedone (Hager et al., 1966).



## 2.6.4 Perchlorate Formation under Nitrifying Conditions

### 2.6.4.1 Background

The potential for  $\text{ClO}_4^-$  generation by nitrifying bacteria was tested in a series of laboratory studies. The goal was to determine if  $\text{ClO}_4^-$  could be produced aerobically under nitrifying conditions from  $\text{ClO}^-$ ,  $\text{ClO}_2^-$ , or  $\text{ClO}_3^-$  as precursors (i.e., like  $\text{NO}_3^-$  is produced from  $\text{NH}_4$  and  $\text{NO}_2^-$  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  $\text{NH}_4$  from  $(\text{NH}_4)_2\text{SO}_4$  as a sole N source) designed for isolation of nitrifiers (Atlas, 1995). Periodically, samples were tested for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  levels via colorimetric test strips. After 3 days of incubation, colorimetric analysis indicated the formation of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in the flask containing sewage sludge. After six days of incubation, colorimetric analysis indicated depletion of  $\text{NO}_2^-$  and formation of  $\text{NO}_3^-$  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  $\text{NO}_2^-$  and  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  formation studies. Rates and extents of  $\text{NO}_3^-$  formation were highest in this media. The Winogradsky's medium was supplemented with 2 mg/L  $\text{ClO}_3^-$ , 2mg/L  $\text{ClO}_2^-$ , 2 mg/L  $\text{ClO}^-$ , 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  $\text{NH}_4$ -free Winogradsky's medium (i.e., Winogradsky's medium made as described above except that  $(\text{NH}_4)_2\text{SO}_4$  was omitted) supplemented with 2 mg/L  $\text{ClO}_3^-$ , 2mg/L  $\text{ClO}_2^-$ , 2 mg/L  $\text{ClO}^-$ , 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) ( $\text{Cl}^-$ ,  $\text{NO}_2^-$  (as N),  $\text{SO}_4^-$ ,  $\text{NO}_3^-$  (as N),  $\text{ClO}_3^-$ ,  $\text{PO}_4^{2-}$ , and  $\text{ClO}_2^-$ ) via EPA Method 300.0.

### 2.6.4.3 Results and Discussion

Significant  $\text{NO}_3^-$  formation was observed in unamended Winogradsky's medium, medium supplemented with 2 mg/L  $\text{ClO}_3^-$ , and  $\text{Cl}^-$ - free medium. In these three treatments,  $\text{NO}_3^-$  levels increased from  $\sim 45$  mg/L ( $\pm 0.6$ ;  $n=3$ ) to 287 mg/L ( $\pm 2$ ;  $n=3$ ) in two weeks, confirming that nitrification was occurring. Low-level  $\text{NO}_3^-$  formation was also observed in  $\text{NH}_4$ -free Winogradsky's medium supplemented with 2 mg/L  $\text{ClO}_3^-$  or 2 mg/L  $\text{ClO}_2^-$ ; in these treatments

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

The loss of  $\text{ClO}_3^-$  under aerobic, nitrifying conditions is interesting. The only chlorine oxyanion that is more oxidized than  $\text{ClO}_3^-$  is  $\text{ClO}_4^-$ , but no  $\text{ClO}_4^-$  was generated during the reaction. It is possible that the loss of  $\text{ClO}_3^-$  in the system was reductive (i.e., the  $\text{ClO}_3^-$  was reduced to chloride), and coupled to the oxidation of  $\text{NH}_4$  or  $\text{NO}_2^-$ . 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  $\text{Cl}^-$  and  $\text{O}$ ) any  $\text{ClO}_2^-$  formed during  $\text{ClO}_3^-$  reduction, and thus mitigate toxicity of this product. Aerobic destruction of  $\text{ClO}_3^-$  may be important for understanding the mass balance of chlorine oxyanions (e.g., concentrations of  $\text{ClO}_3^-$  vs  $\text{ClO}_4^-$ ) in arid environments if this process occurs routinely. However, the absence of any  $\text{ClO}_4^-$  generation suggests that nitrifying strains do not produce  $\text{ClO}_4^-$  from  $\text{Cl}^-$ ,  $\text{ClO}_2^-$  or  $\text{ClO}_3^-$ , 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  $\text{Na}_2\text{S}_2\text{O}_3$  to  $\text{SO}_4$ , 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  $\text{ClO}_3^-$  to  $\text{ClO}_4^-$ .

### 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](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  $\text{O}_3$ -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.**

| <b>Variation #</b> | <b>O<sub>3</sub>-containing compound (5 mM)</b> | <b>Perchlorate precursor (0.1 mM)</b> |
|--------------------|-------------------------------------------------|---------------------------------------|
| 1                  | Sodium thiosulfate                              | Chlorate (as sodium chlorate)         |
| 2                  | Sodium thiosulfate                              | Chlorite (as sodium chlorite)         |
| 3                  | Sodium sulfite                                  | Chlorate (as sodium chlorate)         |
| 4                  | Sodium sulfite                                  | Chlorite (as sodium chlorite)         |
| 5                  | Sodium phosphite                                | Chlorate (as sodium chlorate)         |
| 6                  | Sodium phosphite                                | Chlorite (as sodium chlorite)         |
| 7                  | None                                            | Chlorate (as sodium chlorate)         |
| 8                  | None                                            | Chlorite (as sodium chlorite)         |
| 9                  | Sodium thiosulfate                              | None                                  |
| 10                 | Sodium sulfite                                  | None                                  |
| 11                 | Sodium phosphite                                | None                                  |

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 ClO<sub>4</sub><sup>-</sup> 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 ClO<sub>4</sub><sup>-</sup> 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 ClO<sub>4</sub><sup>-</sup> analysis are provided in Table 2.6.7. The data showed no significantly enhanced ClO<sub>4</sub><sup>-</sup> production in any set of live samples compared to uninoculated controls.

To confirm these results, (i.e., that no significant ClO<sub>4</sub><sup>-</sup> 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 ClO<sub>4</sub><sup>-</sup> generation was documented.

**Table 2.6.7. Perchlorate concentrations ( $\mu\text{g/L}$ ) in studies with *Starkeya novella* and TSP69.**

| Time      |      | Media components |         |          |          | Inoculants |                         |              |
|-----------|------|------------------|---------|----------|----------|------------|-------------------------|--------------|
| date      | days | thiosulfate      | sulfite | chlorate | chlorite | TSP 69     | <i>Starkeya novella</i> | Uninoculated |
| 2/15/2012 | 7    | X                |         | X        |          | ND         | ND                      | ND           |
| 2/22/2012 | 14   | X                |         | X        |          | 0.145      | 0.152                   | 0.123        |
| 2/29/2012 | 21   | X                |         | X        |          | 0.223      | 0.246                   | 0.247        |
| 3/8/2012  | 29   | X                |         | X        |          | 0.344      | 0.339                   | 0.341        |
| 2/15/2012 | 7    | X                |         |          | X        | ND         | ND                      | 0.044        |
| 2/22/2012 | 14   | X                |         |          | X        | 0.041      | 0.029                   | 0.027        |
| 2/29/2012 | 21   | X                |         |          | X        | 0.085      | 0.088                   | 0.096        |
| 3/8/2012  | 29   | X                |         |          | X        | 0.241      | 0.190                   | 0.176        |
| 2/15/2012 | 7    |                  | X       | X        |          | 0.108      | ND                      | 0.100        |
| 2/22/2012 | 14   |                  | X       | X        |          | 0.130      | 0.141                   | 0.132        |
| 2/29/2012 | 21   |                  | X       | X        |          | 0.238      | 0.239                   | 0.226        |
| 3/8/2012  | 29   |                  | X       | X        |          | 0.368      | 0.356                   | 0.285        |
| 2/15/2012 | 7    |                  |         | X        |          | 0.096      | 0.088                   | ND           |
| 2/22/2012 | 14   |                  |         | X        |          | 0.147      | 0.140                   | 0.138        |
| 2/29/2012 | 21   |                  |         | X        |          | 0.259      | 0.262                   | 0.264        |
| 3/8/2012  | 29   |                  |         | X        |          | 0.296      | 0.341                   | 0.361        |
| 2/15/2012 | 7    |                  |         |          | X        | 0.064      | 0.050                   | 0.050        |
| 2/22/2012 | 14   |                  |         |          | X        | 0.041      | 0.038                   | 0.029        |
| 2/29/2012 | 21   |                  |         |          | X        | ND         | 0.077                   | 0.093        |
| 3/8/2012  | 29   |                  |         |          | X        | 0.146      | 0.197                   | 0.173        |
| 2/15/2012 | 7    | X                |         |          |          | 0.067      | 0.040                   | 0.036        |
| 2/22/2012 | 14   | X                |         |          |          | 0.029      | 0.008                   | 0.014        |
| 2/29/2012 | 21   | X                |         |          |          | 0.056      | 0.052                   | 0.050        |
| 3/8/2012  | 29   | X                |         |          |          | 0.067      | 0.092                   | 0.047        |

## 2.7 Evaluate the Accumulation of $\text{ClO}_4^-$ in Plants and their Role in Ozone-Mediated Production of $\text{ClO}_4^-$

### 2.7.1 $\text{ClO}_4^-$ Accumulation at Environmentally-Relevant $\text{O}_3$ Concentrations.

#### 2.7.1.1 Background

The role of tropospheric  $\text{O}_3$  in the origin of non-anthropogenic  $\text{ClO}_4^-$  remains unclear. Tropospheric  $\text{O}_3$  has increased in concentration since pre-industrial times (Vingarzan, 2004; Stevenson et al., 2006). Current levels of ambient  $\text{O}_3$  are injurious to crop species and to native vegetation (Avnery et al., 2011a,b; Booker et al., 2009; USEPA, 2012). High concentrations of  $\text{O}_3$  have been shown experimentally to produce  $\text{ClO}_4^-$  from  $\text{Cl}^-$  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  $\text{ClO}_4^-$  in the US and Chile exhibits a significant  $\Delta^{17}\text{O}$  anomaly, suggesting some production of natural  $\text{ClO}_4^-$  through  $\text{O}_3$  mediated oxidation reactions. However, other sources appear to have a small  $\text{O}_3$  mediated contribution (Böhlke et al., 2005; Jackson et al., 2010). Our preliminary evidence (Burkey et al., unpublished) shows that  $\text{O}_3$ -sensitive and  $\text{O}_3$ -tolerant genotypes of snap bean (*Phaseolus vulgaris*) accumulate foliar  $\text{ClO}_4^-$  under field conditions, and the role of contrasting  $\text{O}_3$  environments is currently being evaluated. Through abscission and litter turnover this would represent an unaccounted source of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ . These factors, the ubiquitous distribution of plants, and the post-industrial increase in  $\text{O}_3$  exposure are consistent with the possibility that tropospheric  $\text{O}_3$  may induce biosynthesis of  $\text{ClO}_4^-$  from  $\text{Cl}^-$  in plants. This would represent a novel source of  $\text{ClO}_4^-$  in the environment.

We present the results of a series of experimental exposures to environmentally relevant concentrations of  $\text{O}_3$  of a broad range of contrasting food, feed and fiber species under controlled conditions. We test the hypotheses that (1) exposure of plants to  $\text{O}_3$  leads to foliar biosynthesis of  $\text{ClO}_4^-$  in young, physiologically active leaves, that (2) such exposure leads to accumulation of  $\text{ClO}_4^-$  in older, senescing leaves, and that (3) contrasting plant species exhibit little foliar  $\text{ClO}_4^-$  at low  $\text{O}_3$  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<sub>3</sub> and C<sub>4</sub> species.

The C<sub>3</sub> 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<sub>4</sub> species were sorghum (*Sorghum bicolor* cv. 4662, Pioneer Seed Co., Johnston IA), sugarcane (*Saccharum officinarum* 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<sup>-1</sup>, 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  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD; 400 – 700 nm at plant level) near solar noon.

#### **Ozone Exposure**

Plants were exposed to environmentally relevant O<sub>3</sub> 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<sub>3</sub> was provided to the CSTRs by corona discharge (Model SGC-11, Pacific O<sub>3</sub> Technology, Brentwood, CA) from purified oxygen (Series ATF-15, Model 1242, SeQual Technologies Inc., San Diego CA). Feedback for the O<sub>3</sub> generator was provided by the exit

stream of a single exposure chamber, monitored with an ultraviolet O<sub>3</sub> monitor (ThermoElectron Model 41C), with other CSTRs controlled by ratio of O<sub>3</sub> flow rate (Grantz et al., 2010). Each CSTR was monitored every 15 min, independently of the control system, with a separate ThermoElectron Model 41C. All monitors were calibrated against a factory certified calibration unit (Model 306; 2B Technologies; Boulder CO). Air with the desired O<sub>3</sub> 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 ClO<sub>4</sub><sup>-</sup> 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 ClO<sub>4</sub><sup>-</sup>), 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 ClO<sub>4</sub><sup>-</sup>.

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 ClO<sub>4</sub><sup>-</sup>.



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 ( $^{18}\text{O}$ ) labeled  $\text{ClO}_4^-$  internal standard. The method detection limit (MDL) for  $\text{ClO}_4^-$  was 0.01  $\mu\text{M}$ .  $\text{ClO}_4^-$  content of tissue, potting medium, and fertilizer is reported as ( $\mu\text{g (kg dry wt)}^{-1}$ ).  $\text{ClO}_4^-$  content of irrigation water and fertilizer solution is reported as ( $\mu\text{g L}^{-1}$ ).

### ***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  $\text{O}_3$  (Massman and Grantz, 1995) and multiplied by mean  $\text{O}_3$  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  $\text{O}_3$  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  $\text{ClO}_4^-$  were normalized by basal values observed at 4 ppb  $\text{O}_3$  for consideration of the relationship between accumulation and potential sensitivity of  $\text{ClO}_4^-$  contents to  $\text{O}_3$  exposure. For comparison of responses of tissue  $\text{ClO}_4^-$  content to  $\text{O}_3$  exposure vs.  $\text{O}_3$  dose,  $\text{ClO}_4^-$  values were normalized within each species by the median value of  $\text{ClO}_4^-$  across all  $\text{O}_3$  exposures.

Each species was analyzed independently for response to  $\text{O}_3$  exposure. Basal  $\text{ClO}_4^-$  content of all species at low  $\text{O}_3$  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  $\text{ClO}_4^-$  (Table 2.7.1). Similarly, the commercial potting mix exhibited relatively low  $\text{ClO}_4^-$  content. The commercial fertilizer applied to all species contained substantial amounts of  $\text{ClO}_4^-$  on a dry weight basis (Table 2.7.1), but only  $3.6 \mu\text{g ClO}_4^- \text{ L}^{-1}$  in the dilute irrigation solution. Over the approximate 9 weeks of plant growth, each pot received a total of approximately  $4.4 \mu\text{g ClO}_4^-$  from the fertilizer that was applied twice weekly, and an additional  $2.6 \mu\text{g ClO}_4^-$  in irrigation water applied daily. These provided the principal source for  $\text{ClO}_4^-$  accumulation from the growth medium (Table 2.7.1).

#### ***Perchlorate Accumulation***

Large interspecific differences were observed in foliar concentrations of  $\text{ClO}_4^-$  averaged across all  $\text{O}_3$  exposures (Figure 2.7.1; Table 2.7.2). These appear to reflect physiological differences in uptake or exclusion of  $\text{ClO}_4^-$  present in the growth medium. Spinach accumulated approximately  $700 \mu\text{g (kg dry wt)}^{-1}$ , the highest level observed, while sugarcane accumulated less than  $100 \mu\text{g kg}^{-1}$ . Other species were intermediate, with young leaf contents lying generally between  $150$ - $250 \mu\text{g kg}^{-1}$ . On average, plants accumulated about 7% of the applied  $\text{ClO}_4^-$  over their lifespan, much of the remainder being lost to drainage.

The results were similar for older, senescing leaves (Table 2.7.3) although  $\text{ClO}_4^-$  was accumulated to much higher levels in these leaves. Young leaves of broccoli exhibited contents near  $400 \mu\text{g kg}^{-1}$  (Table 2.7.2) whereas older leaves averaged slightly over  $1000 \mu\text{g kg}^{-1}$  (Table 2.7.3). For sugarcane, the corresponding values were about  $50 \mu\text{g kg}^{-1}$  for young leaves (Table 2.7.2) but  $>100 \mu\text{g kg}^{-1}$  for older leaves (Table 2.7.3). Averaged over all species, older leaves accumulated more than twice the  $\text{ClO}_4^-$  as younger leaves ( $>500 \mu\text{g kg}^{-1}$  vs.  $<250 \mu\text{g kg}^{-1}$ ).

#### ***Effect of Ozone Exposure on Perchlorate Accumulation***

There was no consistent effect of  $\text{O}_3$  exposure on foliar content of  $\text{ClO}_4^-$  in young leaves. Five of nine species exhibited a decline in  $\text{ClO}_4^-$  content with increasing  $\text{O}_3$  exposure while for the remaining four species  $\text{ClO}_4^-$  content increased with  $\text{O}_3$  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  $\text{O}_3$  exposure (Table 2.7.2). The same absence of relationship with  $\text{O}_3$  exposure was observed in older leaves, despite the greater overall accumulation of  $\text{ClO}_4^-$  (Table 2.7.3).

Basal accumulation of  $\text{ClO}_4^-$  at low  $\text{O}_3$  was not predictive of the effect of elevated  $\text{O}_3$ , on  $\text{ClO}_4^-$  in young leaves, whether significant or not (Figure 2.7.2; Table 2.7.4). This was the case for absolute changes in  $\text{ClO}_4^-$  between low and moderate  $\text{O}_3$  and between low and higher  $\text{O}_3$

exposures (Figure 2.7.2A; triangles, squares, respectively). The relationship was not improved by normalization of the values associated with O<sub>3</sub> exposure by basal ClO<sub>4</sub><sup>-</sup> content (Figure 2.7.2B).

We considered whether O<sub>3</sub>-induced foliar biosynthesis of ClO<sub>4</sub><sup>-</sup> might occur, yet not be reflected in leaf contents due to transport to the rhizosphere. However, there was no relationship between ClO<sub>4</sub><sup>-</sup> in the top level of the potting medium and basal accumulation of ClO<sub>4</sub><sup>-</sup> at 4 ppb O<sub>3</sub> (Table 2.7.4). Similarly, there was no significant relationship between O<sub>3</sub> exposure of plant and potting medium and surface content of ClO<sub>4</sub><sup>-</sup> (Table 2.7.4). However, a multiple range test of ClO<sub>4</sub><sup>-</sup> in the surface layer of the growth medium of unused potting medium, potting medium exposed at 4 ppb O<sub>3</sub>, and potting medium exposed at 114 ppb O<sub>3</sub> (Table 2.7.5) suggested a positive association between ClO<sub>4</sub><sup>-</sup> and O<sub>3</sub>. The potting medium exposed to the highest and lowest O<sub>3</sub> concentrations did not differ in ClO<sub>4</sub><sup>-</sup> content, and that exposed to the lowest O<sub>3</sub> did not differ from the unused medium. However, there was a significant difference between unused potting medium and that exposed to the highest O<sub>3</sub> concentration.

**Table 2.7.1.  $\text{ClO}_4^-$  content of unused irrigation water, potting medium, and fertilizer.**

| <b>Material</b>       | <b>n</b> | <b>Perchlorate Content</b>                 | <b>S.E.</b> | <b>Range</b> |
|-----------------------|----------|--------------------------------------------|-------------|--------------|
| Irrigation Water      | 11       | 0.61<br>( $\mu\text{g L}^{-1}$ )           | $\pm 0.053$ | 0.33 – 0.81  |
| Potting Medium        | 12       | 3.78<br>( $\mu\text{g (kg dry wt)}^{-1}$ ) | $\pm 0.72$  | 0.43-6.41    |
| Fertilizer (granular) | 6        | 1270<br>( $\mu\text{g (kg dry wt)}^{-1}$ ) | $\pm 89.3$  | 1090-1700    |
| (asapplied)           |          | 3.55<br>( $\mu\text{g L}^{-1}$ )           | $\pm 0.25$  |              |

**Table 2.7.2. Perchlorate content ( $\mu\text{g kg}^{-1}$ ) of young leaves of a range of crop species as a function of  $\text{O}_3$  exposure (ppb, 12 hr mean) sampled at the time of final harvest.**  
There were no significant differences between  $\text{O}_3$  exposures ( $P < 0.05$ ) within any species, nor within the pooled data.

| Species     | n  | $\text{O}_3$ Exposure (ppb) | Perchlorate Content ( $\mu\text{g kg}^{-1}$ ) | S.E.        | Range     |
|-------------|----|-----------------------------|-----------------------------------------------|-------------|-----------|
| Bush Bean   | 6  | 4                           | 155                                           | $\pm 31.3$  | 72.1-294  |
|             | 6  | 59                          | 160                                           | $\pm 19.0$  | 110-225   |
|             | 6  | 114                         | 204                                           | $\pm 15.4$  | 130-236   |
| Broccoli    | 9  | 4                           | 380                                           | $\pm 41.4$  | 234-653   |
|             | 7  | 59                          | 349                                           | $\pm 75.0$  | 42.1-672  |
|             | 9  | 114                         | 528                                           | $\pm 86.1$  | 68.3-927  |
| Lettuce     | 9  | 4                           | 223                                           | $\pm 89.0$  | 65.8-926  |
|             | 9  | 59                          | 145                                           | $\pm 26.3$  | 56.2-279  |
|             | 9  | 114                         | 151                                           | $\pm 20.1$  | 81.3-250  |
| Maize       | 6  | 4                           | 173                                           | $\pm 54.1$  | 15.0-371  |
|             | 5  | 59                          | 85                                            | $\pm 10.2$  | 50.1-114  |
|             | 6  | 114                         | 123                                           | $\pm 38.3$  | 23.9-295  |
| Pima Cotton | 18 | 4                           | 290                                           | $\pm 40.2$  | 33.4-544  |
|             | 18 | 59                          | 218                                           | $\pm 24.8$  | 75.5-416  |
|             | 18 | 114                         | 218                                           | $\pm 36.3$  | 58.6-590  |
| Sorghum     | 6  | 4                           | 177                                           | $\pm 38.4$  | 87.5-328  |
|             | 6  | 59                          | 182                                           | $\pm 20.6$  | 128-267   |
|             | 5  | 114                         | 369                                           | $\pm 110.3$ | 84.2-598  |
| Soybean     | 5  | 4                           | 222                                           | $\pm 39.8$  | 136-366   |
|             | 6  | 59                          | 218                                           | $\pm 16.8$  | 164-284   |
|             | 6  | 114                         | 186                                           | $\pm 24.7$  | 123-294   |
| Spinach     | 3  | 4                           | 766                                           | $\pm 84.5$  | 670-934   |
|             | 2  | 59                          | 568                                           | $\pm 110.6$ | 458-679   |
|             | 3  | 114                         | 686                                           | $\pm 31.8$  | 622-719   |
| Sugarcane   | 6  | 4                           | 42.5                                          | $\pm 6.2$   | 30.6-71.4 |
|             | 6  | 59                          | 61.0                                          | $\pm 19.0$  | 6.67-132  |
|             | 6  | 114                         | 54.6                                          | $\pm 15.3$  | 15.0-122  |
| All Species | 68 | 4                           | 255                                           | $\pm 24.8$  | 15.00-934 |
|             | 65 | 59                          | 199                                           | $\pm 17.0$  | 6.67-679  |
|             | 68 | 114                         | 255                                           | $\pm 26.1$  | 15.0-927  |

**Table 2.7.3. Perchlorate content of older, senescing leaves of a range of crop species as a function of O<sub>3</sub> exposure (ppb, 12 hr mean) sampled at the time of final harvest.**

There were no significant differences between O<sub>3</sub> exposures ( $P < 0.05$ ) within any species, nor within the pooled data.

| <b>Species</b> | <b>n</b> | <b>O<sub>3</sub><br/>Exposure<br/>(ppb)</b> | <b>Perchlorate<br/>Content<br/>(µg kg<sup>-1</sup>)</b> | <b>S.E.</b> | <b>Range</b> |
|----------------|----------|---------------------------------------------|---------------------------------------------------------|-------------|--------------|
| Broccoli       | 9        | 4                                           | 1730                                                    | ± 970       | 312-9450     |
|                | 8        | 59                                          | 765                                                     | ± 123       | 423-1430     |
|                | 8        | 114                                         | 945                                                     | ± 170       | 417-1960     |
| Lettuce        | 2        | 4                                           | 337                                                     | ± 188       | 150-525      |
|                | 3        | 59                                          | 430                                                     | ± 171       | 165-750      |
|                | 9        | 114                                         | 319                                                     | ± 62.5      | 188-750      |
| Maize          | 6        | 4                                           | 87.0                                                    | ± 19.8      | 51.5-184     |
|                | 6        | 59                                          | 85.1                                                    | ± 14.0      | 17.4-110     |
|                | 5        | 114                                         | 77.4                                                    | ± 11.6      | 54.9-120     |
| Pima Cotton    | 12       | 4                                           | 435                                                     | ± 133       | 97.5-1320    |
|                | 17       | 59                                          | 352                                                     | ± 74.6      | 78.1-1280    |
|                | 18       | 114                                         | 549                                                     | ± 170       | 70.3-2330    |
| Sorghum        | 3        | 4                                           | 238                                                     | ± 11.8      | 214-250      |
|                | 3        | 59                                          | 527                                                     | ± 166.4     | 348-860      |
|                | 3        | 114                                         | 463                                                     | ± 77.3      | 375-617      |
| Sugarcane      | 3        | 4                                           | 131                                                     | ± 22.7      | 104-176      |
|                | 3        | 59                                          | 85.7                                                    | ± 16.0      | 58.4-114     |
|                | 6        | 114                                         | 95.4                                                    | ± 7.94      | 72.1-125     |
| All Species    | 35       | 4                                           | 660                                                     | ± 267       | 51.5-9450    |
|                | 40       | 59                                          | 394                                                     | ± 55.2      | 17.4-1432    |
|                | 49       | 114                                         | 462                                                     | ± 78.7      | 54.9-2330    |

**Table 2.7.4. Perchlorate content ( $\mu\text{g (kg dry wt)}^{-1}$ ) of potting medium after use for plant growth by a range of crop species, sampled at the time of final harvest (mean  $\pm$  S.E.), as a function of  $\text{O}_3$  exposure (ppb, 12 hr mean). There were no significant differences between  $\text{O}_3$  exposures ( $P < 0.05$ ) within any species, nor within the pooled data.**

| Species     | n  | $\text{O}_3$<br>Exposure<br>(ppb) | Perchlorate<br>Content<br>( $\mu\text{g kg}^{-1}$ ) | S.E.       | Range      |
|-------------|----|-----------------------------------|-----------------------------------------------------|------------|------------|
| Broccoli    | 6  | 4                                 | 5.68                                                | $\pm 0.40$ | 5.00-7.61  |
|             | 6  | 59                                | 6.09                                                | $\pm 0.26$ | 5.38-7.07  |
|             | 6  | 114                               | 6.2                                                 | $\pm 0.39$ | 4.62-7.31  |
| Bush Bean   | 3  | 4                                 | 6.98                                                | $\pm 0.08$ | 6.88-7.14  |
|             | 3  | 59                                | 6.53                                                | $\pm 0.2$  | 6.15-6.82  |
|             | 3  | 114                               | 6.14                                                | $\pm 0.25$ | 5.88-6.64  |
| Lettuce     | 6  | 4                                 | 6.13                                                | $\pm 0.27$ | 5.49-7.34  |
|             | 6  | 59                                | 5.7                                                 | $\pm 0.21$ | 5.07-6.36  |
|             | 6  | 114                               | 7.32                                                | $\pm 1.08$ | 6.00-12.63 |
| Maize       | 3  | 4                                 | 0.51                                                | $\pm 0.05$ | 0.43-0.60  |
|             | 1  | 59                                | 2.8                                                 | na         | 2.80-2.80  |
|             | 3  | 114                               | 0.44                                                | $\pm 0.06$ | 0.37-0.55  |
| Pima Cotton | 9  | 4                                 | 1.38                                                | 0.29       | 0.65-2.87  |
|             | 8  | 59                                | 3.44                                                | 2.02       | 0.69-17.5  |
|             | 9  | 114                               | 4.49                                                | 2.03       | 1.03-18.6  |
| Sorghum     | 3  | 4                                 | 7.79                                                | $\pm 0.88$ | 6.70-9.52  |
|             | 3  | 59                                | 7.16                                                | $\pm 0.09$ | 6.98-7.25  |
|             | 3  | 114                               | 8.65                                                | $\pm 1.32$ | 7.32-11.3  |
| Soybean     | 3  | 4                                 | 6.96                                                | $\pm 0.31$ | 6.47-7.54  |
|             | 3  | 59                                | 6.65                                                | $\pm 0.61$ | 5.54-7.65  |
|             | 3  | 114                               | 6.41                                                | $\pm 0.25$ | 5.95-6.82  |
| Spinach     | 3  | 4                                 | 5.73                                                | $\pm 0.21$ | 5.34-6.07  |
|             | 3  | 59                                | 6.05                                                | $\pm 0.87$ | 4.59-7.60  |
|             | 3  | 114                               | 6.26                                                | $\pm 0.17$ | 5.93-6.49  |
| Sugarcane   | 3  | 4                                 | 10.7                                                | $\pm 3.18$ | 4.53-15.1  |
|             | 3  | 59                                | 7.55                                                | $\pm 0.43$ | 6.81-8.31  |
|             | 3  | 114                               | 9.31                                                | $\pm 1.83$ | 5.70-11.6  |
| All Species | 39 | 4                                 | 5.11                                                | $\pm 0.53$ | 0.43-15.1  |
|             | 36 | 59                                | 5.63                                                | $\pm 0.50$ | 0.69-17.5  |
|             | 39 | 114                               | 5.98                                                | $\pm 0.61$ | 0.37-18.6  |



**Table 2.7.5. Perchlorate content ( $\mu\text{g (kg dry wt)}^{-1}$ ) 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  $\pm$  S.E.), as a function of  $\text{O}_3$  exposure (ppb, 12 hr mean). Means followed by different letters were significantly different ( $P < 0.05$ ).**

| <b><math>\text{O}_3</math><br/>Exposure<br/>(ppb)</b> | <b>n</b> | <b>Perchlorate<br/>Content<br/>(<math>\mu\text{g kg}^{-1}</math>)</b> | <b>S.E.</b> | <b>Range<br/>(<math>\mu\text{g kg}^{-1}</math>)</b> |
|-------------------------------------------------------|----------|-----------------------------------------------------------------------|-------------|-----------------------------------------------------|
| na                                                    | 12       | 3.78a                                                                 | $\pm 0.72$  | 0.43-6.41                                           |
| 4                                                     | 39       | 5.11ab                                                                | $\pm 0.53$  | 0.43-15.1                                           |
| 114                                                   | 39       | 5.98b                                                                 | $\pm 0.61$  | 0.37-18.6                                           |

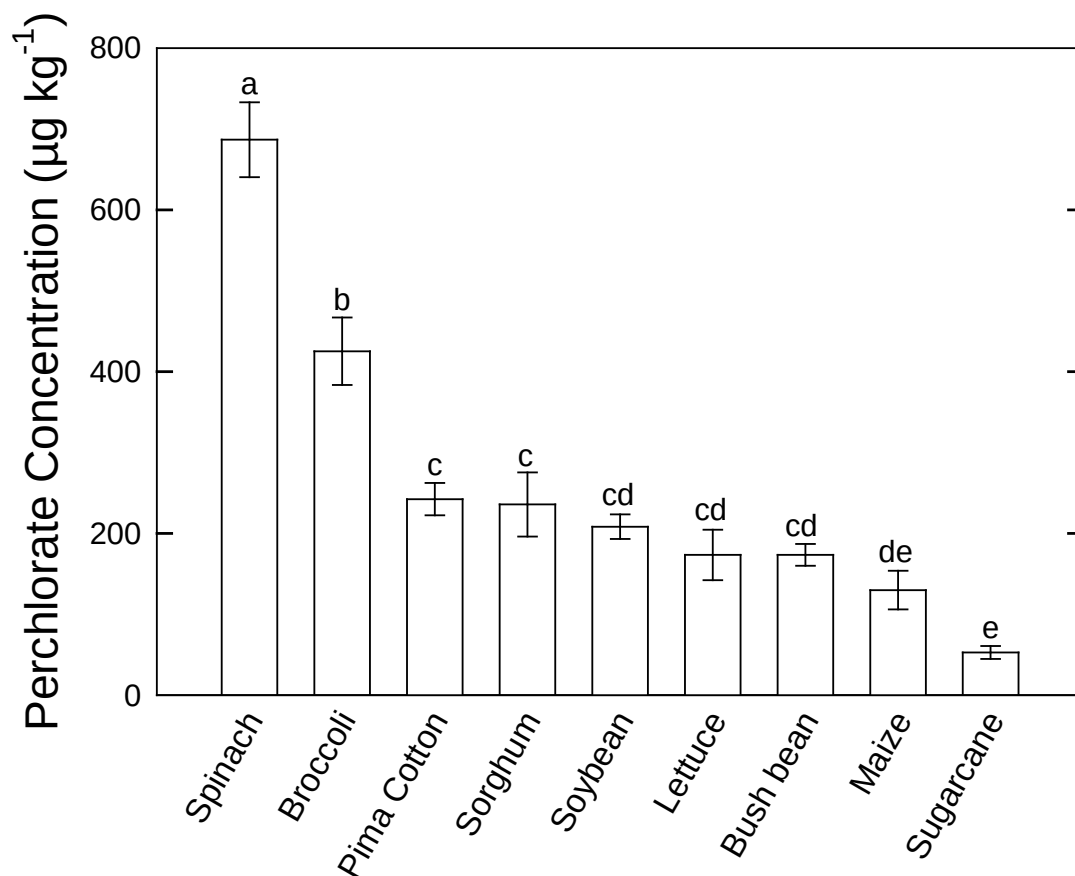


**Figure 2.7.1. Inter-specific differences in perchlorate content ( $\mu\text{g (kg dry wt)}^{-1}$ ) of youngest fully expanded leaves of a range of crop species (mean over all  $\text{O}_3$  exposures  $\pm$  SE). Bars with different letters differ at  $P < 0.05$ .**

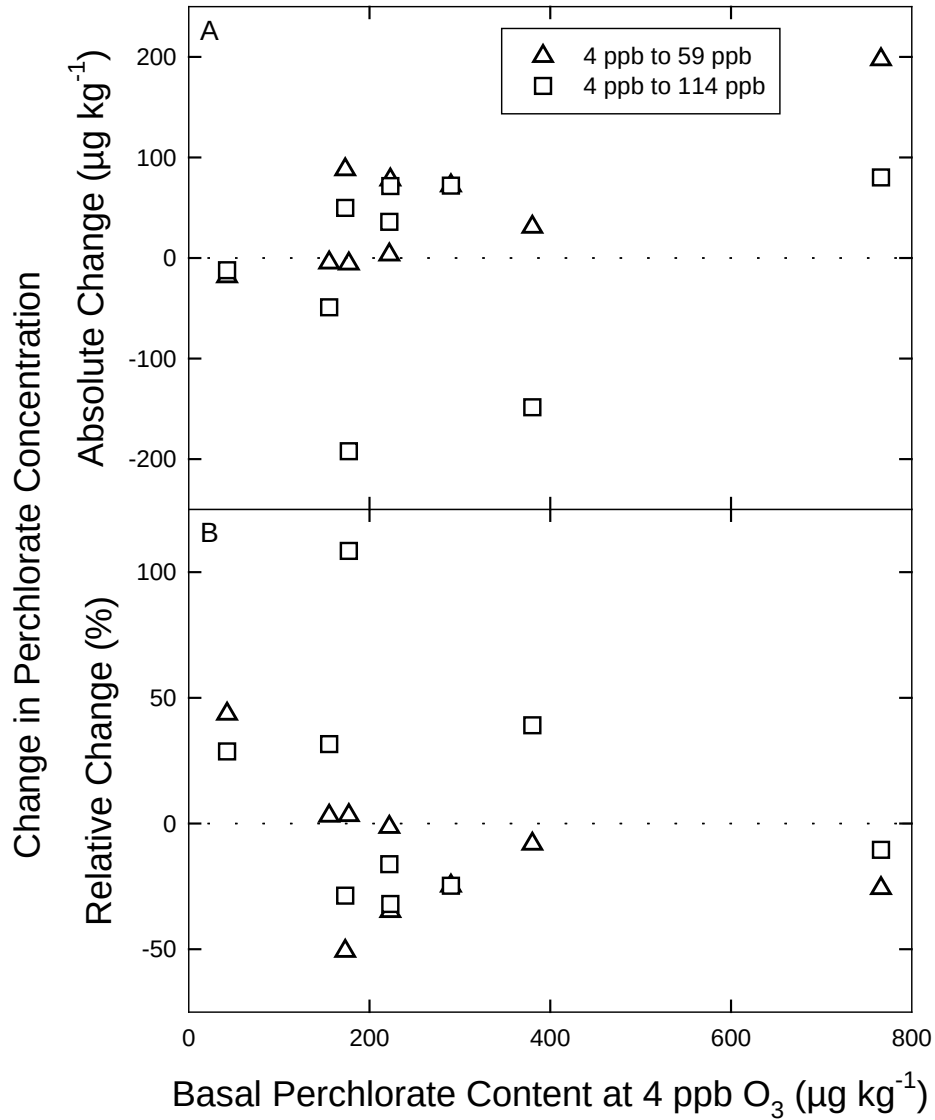


**Figure 2.7.2. There was no significant relationship between basal perchlorate content (at 4 ppb O<sub>3</sub>) and the change in ClO<sub>4</sub><sup>-</sup> content (unitless) between 4 and 59 ppb O<sub>3</sub> (circles) and between 4 and 114 ppb (squares) of youngest fully expanded leaves of a range of crop species.**

### ***Role of Ozone Metrics***

We evaluated whether the lack of response of foliar  $\text{ClO}_4^-$  content in young and older leaves to  $\text{O}_3$  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  $\text{O}_3$  exposure (data not shown).

We also considered whether the use of  $\text{O}_3$  concentration could be an inadequate metric of  $\text{O}_3$  exposure. The lack of relationship between foliar  $\text{ClO}_4^-$  and  $\text{O}_3$  was not improved by use of cumulative stomatal uptake of  $\text{O}_3$  ( $\text{O}_3$  dose) rather than  $\text{O}_3$  concentration ( $\text{O}_3$  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  $\text{ClO}_4^-$  and  $\text{O}_3$  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  $\text{ClO}_4^-$  was considered as a function of  $\text{O}_3$  dose (Fig. 2.7.3B).

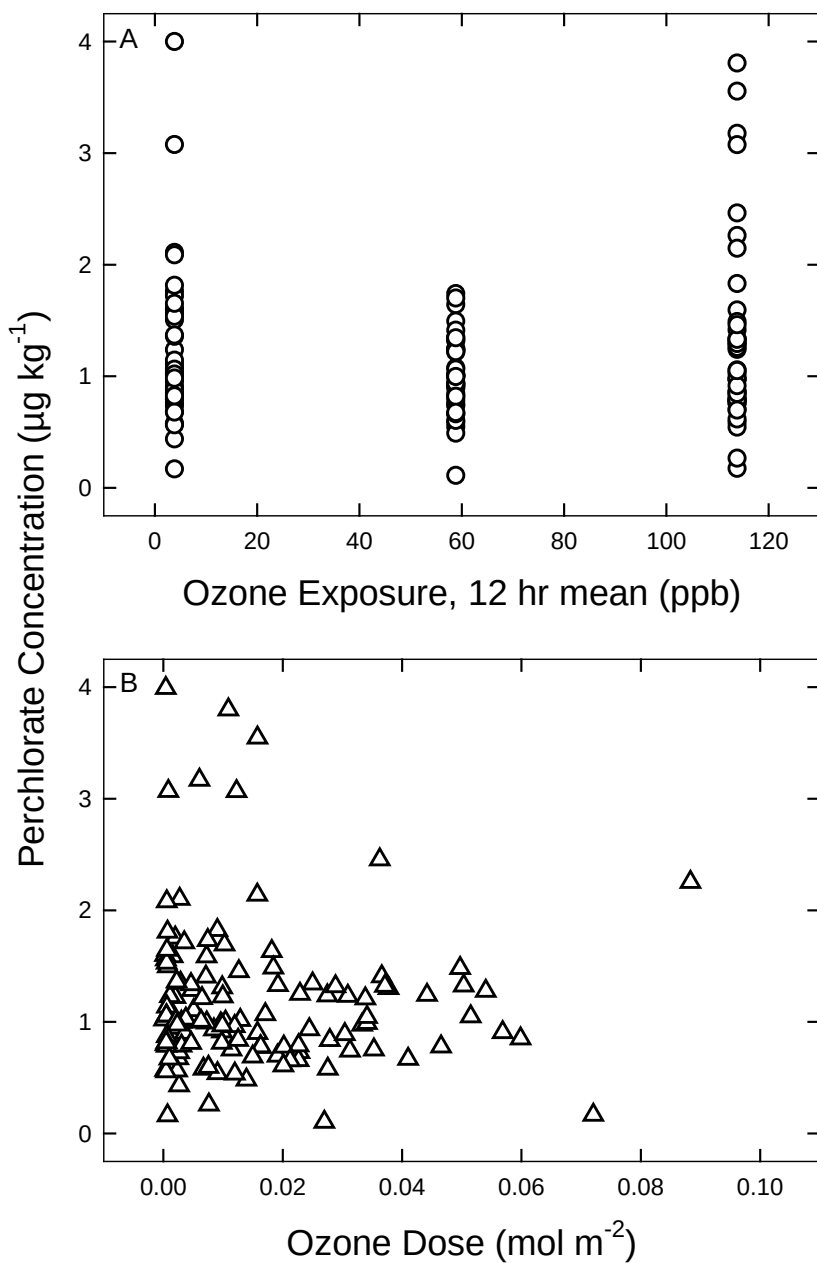


**Figure 2.7.3.** There was no significant relationship between  $\text{O}_3$  exposure (A;  $r^2 = 0.0034$ ) or  $\text{O}_3$  dose (cumulative flux; B;  $r^2=0.0018$ ) and  $\text{ClO}_4^-$  content of young leaves normalized by the median  $\text{ClO}_4^-$  content of each species shown in Figure 2.7.1 for which  $\text{O}_3$  flux data were available.

#### 2.7.1.4 Discussion

##### **Perchlorate Accumulation**

The plants in the current study exhibited a range of accumulation factors for  $\text{ClO}_4^-$  in the growth medium. This is consistent with previous studies showing that plants accumulate  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $700 \mu\text{g kg}^{-1}$ ) are consistent with previous reports for leafy green food crops.

In our study this  $\text{ClO}_4^-$  was contributed mostly by the commercial fertilizer. Although  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  (Tan et al., 2004; Yu et al., 2004; Jackson et al., 2005), with the magnitude of uptake related to the distance from streams draining  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  present in the growth medium. Phytoaccumulation of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ , 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  $\text{ClO}_4^-$  as younger leaves. This represents a potent mechanism for concentrating  $\text{ClO}_4^-$  from the rhizosphere to the soil surface.

### ***Effect of Ozone Exposure on Perchlorate Accumulation***

$\text{O}_3$  exposure had a nearly random effect on foliar content of  $\text{ClO}_4^-$  in both young and older leaves. Approximately half of experimental species exhibited a decline in  $\text{ClO}_4^-$  content with increasing  $\text{O}_3$  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  $\text{O}_3$  effects were not successful. Basal accumulation of  $\text{ClO}_4^-$ , indicative of favorable uptake / unfavorable exclusion properties of root membranes, was not predictive of the effect of  $\text{O}_3$  on  $\text{ClO}_4^-$  content of young leaves. This putative relationship was tested at moderate and at higher  $\text{O}_3$  exposures without success. Similarly, various normalization procedures, seeking to remove the undue influence of high baseline values of  $\text{ClO}_4^-$  in the accumulating species did not improve relationships between  $\text{O}_3$  exposure and changes in tissue  $\text{ClO}_4^-$ .

The  $\text{O}_3$  exposure protocol and its representation as  $\text{O}_3$  concentration was adequate to induce a substantial decline in above ground biomass in these plants, suggesting that the test could have identified  $\text{O}_3$ -induced biosynthesis of  $\text{ClO}_4^-$  if it had occurred. The uptake of  $\text{ClO}_4^-$  observed in this study is not without potential consequence for plants. Millimolar concentrations of  $\text{ClO}_4^-$  in irrigation water reduced photosynthetic electron transport and induced antioxidant metabolism in tobacco and *Arabidopsis* (Hamissou 2011). As  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ , 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  $\text{O}_3$  exposure is not a consequence of enhanced tissue accumulation of  $\text{ClO}_4^-$ , but rather reflects the phytotoxicity of  $\text{O}_3$ , itself. The data were clear in indicating that ambient and near ambient concentrations of  $\text{O}_3$  did not lead to increased tissue contents of  $\text{ClO}_4^-$ .

### ***Alternative Sinks***

We observed bioconcentration of  $\text{ClO}_4^-$  in older, senescing and abscised leaves. In more mature canopy conditions this would serve to shed  $\text{ClO}_4^-$  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  $\text{Cl}^-$  in the irrigation water or fertilizer were exposed directly to  $\text{O}_3$  in the CSTRs. There was some indication that direct exposure of the growth medium to the highest  $\text{O}_3$  concentration may have led to accumulation of  $\text{ClO}_4^-$  in the surface layer. Averaged over all species there was a modest but non-significant relationship with increasing  $\text{O}_3$  exposure, but a significant difference between unused potting medium and medium exposed to the highest  $\text{O}_3$ . This suggests that  $\text{ClO}_4^-$  content in the soil surface may increase with exposure to  $\text{O}_3$ , presumably due to oxidation of  $\text{Cl}^-$  in the soil, as demonstrated experimentally at higher  $\text{O}_3$  concentrations with  $\text{Cl}^-$  coated sand (Kang et al., 2008) and soil (Dasgupta et al., 2005). Our results were observed without artificial enhancement of the  $\text{Cl}^-$  content, at near ambient  $\text{O}_3$  concentrations, and over relatively brief exposure periods relative to geologic time. This potentially important conclusion requires confirmation, but if reproducible and applicable under field conditions, this mechanism would contribute to  $\text{ClO}_4^-$  present in the environment.

The clear absence of  $\text{O}_3$ -sensitivity of  $\text{ClO}_4^-$  in young leaves could have indicated a robust translocation to roots and exudation into the rhizosphere. However, we observed no relationship between foliar accumulation of  $\text{ClO}_4^-$  and its content in the potting medium. Retranslocation to stems or roots has not been detected in previous studies (Vogt and Jackson, 2010), consistent with high concentrations of  $\text{ClO}_4^-$  in leaf laminae and considerably lower concentrations in the rest of the plant. This was the case in the current study, and in *Polygonum spp.* (smartweed) in which leaves accumulated up to  $800 \mu\text{g (kg dwt)}^{-1}$ , while roots and stems accumulated only  $100\text{--}150 \mu\text{g (kg dwt)}^{-1}$  (Tan et al., 2006). Neither exudation to non-contaminated media nor reductive metabolism are significant sinks for phytoaccumulated  $\text{ClO}_4^-$ , (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 ClO<sub>4</sub><sup>-</sup> Accumulation at Elevated O<sub>3</sub> Concentrations

### 2.7.2.1 Background

In the previous Section (2.7.1), we demonstrated that low to moderate concentrations of O<sub>3</sub> (0–114 nL L<sup>-1</sup>, 12 hr mean) did not increase foliar ClO<sub>4</sub><sup>-</sup>, despite large interspecific differences in ClO<sub>4</sub><sup>-</sup> uptake from the growth medium. However, these previous data did not disprove the hypothesis that much higher concentrations of O<sub>3</sub>, such as that observed during stratosphere-troposphere folding events or during O<sub>3</sub> episodes in emerging mega-cities, might stimulate O<sub>3</sub> synthesis or accumulation in plants. Here we test the hypothesis that exposure of plants to environmentally plausible, but very high, concentrations of O<sub>3</sub> may lead to accumulation of ClO<sub>4</sub><sup>-</sup> 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 C<sub>3</sub> 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 C<sub>4</sub> 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 O<sub>3</sub>, 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<sup>-1</sup>, Miracle Gro, Scotts Miracle-Gro Products Inc., Port Washington, NY). These plants were exposed to relatively low amounts of ClO<sub>4</sub><sup>-</sup> in their growth environment. The ClO<sub>4</sub><sup>-</sup> content of the fertilizer was 3.55 ± 0.25 µg L<sup>-1</sup>, and that of the potting soil and irrigation water were 3.77 ± 0.72 µg and 0.61 ± 0.05 µg L<sup>-1</sup> (kg dry wt)<sup>-1</sup>, 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 ClO<sub>4</sub><sup>-</sup>, the principal substrate for ClO<sub>4</sub><sup>-</sup> 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<sup>-2</sup> s<sup>-1</sup> PPFD at plant level, reflecting shading by both the greenhouse structure and the CSTR. Air with appropriate O<sub>3</sub> concentrations (12 hour means nominally 4, 102, and 204 nL L<sup>-1</sup>; daily maxima near solar noon

of 4, 160 and 320 nL L<sup>-1</sup>) was introduced at one complete air exchange per minute into each CSTR.

O<sub>3</sub> was produced by corona discharge (Model SGC-11, Pacific O<sub>3</sub> Technology, Brentwood, CA) from a feedstock of purified oxygen (Series ATF-15, Model 1242, SeQual Technologies Inc., San Diego CA). O<sub>3</sub> concentration followed a half-sine wave during daylight hours, 7 days week<sup>-1</sup>, with voltage to the O<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> flux, conductance was converted from water vapor to O<sub>3</sub> using the ratio of diffusivities (Massman and Grantz, 1995; Massman, 1998), and multiplied by mean O<sub>3</sub> concentration during the 2 hour period centered on the conductance measurement. O<sub>3</sub> 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  $\text{ClO}_4^-$  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  $1.0 \pm 0.1$  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  $\text{ClO}_4^-$ ), and an extraction blank (DDI water only). Samples were spiked with an oxygen-isotope ( $^{18}\text{O}$ ) labeled  $\text{ClO}_4^-$  internal standard, then loaded into a 25  $\mu\text{L}$  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  $0.3 \text{ ml min}^{-1}$ ). The method detection limit (MDL) was  $0.01 \mu\text{M ClO}_4^-$ .

### ***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  $\text{O}_3$  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  $\text{ClO}_4^-$  and  $\text{O}_3$  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  $\text{ClO}_4^-$  and  $\text{O}_3$  dose was evaluated similarly, using both native  $\text{ClO}_4^-$  data and  $\text{ClO}_4^-$  data normalized within each species by the median value of  $\text{ClO}_4^-$  determined in each species over all  $\text{O}_3$  exposures.

Basal  $\text{ClO}_4^-$  content of all species at low  $\text{O}_3$  concentration was subjected to independent analysis by ANOVA (PROC GLM and PROC MEANS), with means separation by Duncan's Multiple

Range Test. 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  $\text{ClO}_4^-$  from the rhizosphere (Table 2.7.6). The large capacity to accumulate  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  at low  $\text{O}_3$  ranged from about  $130 \pm 16 \mu\text{g (kg dry wt)}^{-1}$  (range  $87 - 205 \mu\text{g (kg dry wt)}^{-1}$ ) in Pima cotton to over  $500 \pm 191 \mu\text{g (kg dry wt)}^{-1}$  (range  $252 - 1469 \mu\text{g (kg dry wt)}^{-1}$ ) in young leaves of soybean (Table 2.7.6). In our previous study described in Section 2.7.1, spinach (*Spinacia oleracea*) accumulated over  $700 \mu\text{g (kg dry wt)}^{-1}$ , while sugarcane (*Saccharum spp.*, hybrid) accumulated less than  $100 \mu\text{g (kg dry wt)}^{-1}$ .

These species differences reflect physiological differences in uptake/exclusion of  $\text{ClO}_4^-$  present in the growth medium. In both studies, plants accumulated less than 10% of the available  $\text{ClO}_4^-$  over the entire study period, but exhibited bioconcentration factors of up to two orders of magnitude. Accumulation of  $\text{ClO}_4^-$  was greater in older than in younger leaves as described in Section 2.7.1, reflecting the greater duration of uptake.  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  than the sheltered inner leaves (Ha et al., 2013). Re-translocation of  $\text{ClO}_4^-$  has not been reported.

Root and stem tissue exhibit very low  $\text{ClO}_4^-$  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  $800 \mu\text{g (kg dry wt)}^{-1}$ , while roots and stems contained less than  $150 \mu\text{g (kg dry wt)}^{-1}$  (Tan et al., 2006).

Accumulation of  $\text{ClO}_4^-$  from the rhizosphere, its sequestration in older leaf cohorts, and their subsequent abscission, represents a possible mechanism of transporting and concentrating rhizosphere  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  values ranged from 6.2 to 8.4  $\mu\text{g (kg dry wt)}^{-1}$  (Table 2.7.7) at the highest  $\text{O}_3$  exposure, similar to the values observed previously at lower  $\text{O}_3$  (Table 2.7.4 in Section 2.7.1 and Table 2.7.7 herein). There was no relationship between plant capacity for  $\text{ClO}_4^-$  accumulation and the  $\text{ClO}_4^-$  content of the surface soil.

**Table 2.7.6. Perchlorate concentration ( $\mu\text{g (kg dry wt)}^{-1}$ ) of young leaves of contrasting crop species exposed to filtered air. Values followed by the same letter do not differ at  $P < 0.05$ .**

| Species     | $\text{O}_3$<br>Exposure<br>( $\text{nL L}^{-1}$ ) | n | Perchlorate<br>Content<br>( $\mu\text{g (kg dry wt)}^{-1}$ ) | S.E.         | Range          |
|-------------|----------------------------------------------------|---|--------------------------------------------------------------|--------------|----------------|
| Soybean     | 4                                                  | 6 | 524.54 <b>a</b>                                              | $\pm 190.88$ | 251.90-1469.00 |
| Bush bean   | 4                                                  | 6 | 307.90 <b>a,b</b>                                            | $\pm 95.60$  | 119.50-678.30  |
| Maize       | 4                                                  | 6 | 160.33 <b>b</b>                                              | $\pm 76.49$  | 30.00-514.10   |
| Sorghum     | 4                                                  | 6 | 134.34 <b>b</b>                                              | $\pm 13.18$  | 90.21-174.10   |
| Pima Cotton | 4                                                  | 6 | 133.86 <b>b</b>                                              | $\pm 16.37$  | 86.64-205.10   |

**Table 2.7.7. Perchlorate concentration ( $\mu\text{g (kg dry wt)}^{-1}$ ) of potting medium used for plant growth by contrasting crop species exposed to high  $\text{O}_3$ . Values followed by the same letter do not differ at  $P < 0.05$ .**

| Species     | $\text{O}_3$<br>Exposure<br>( $\text{nL L}^{-1}$ ) | n | Perchlorate<br>Content<br>( $\mu\text{g (kg dry wt)}^{-1}$ ) | S.E.       | Range      |
|-------------|----------------------------------------------------|---|--------------------------------------------------------------|------------|------------|
| Soybean     | 204                                                | 4 | 8.39 <b>a</b>                                                | $\pm 2.77$ | 4.86-16.64 |
| Bush bean   | 204                                                | 4 | 5.92 <b>a</b>                                                | $\pm 0.48$ | 4.97-7.24  |
| Maize       | 204                                                | 4 | 7.74 <b>a</b>                                                | $\pm 2.07$ | 4.44-13.80 |
| Sorghum     | 204                                                | 4 | 6.89 <b>a</b>                                                | $\pm 1.41$ | 4.53-10.60 |
| Pima Cotton | 204                                                | 4 | 6.20 <b>a</b>                                                | $\pm 0.18$ | 5.84-6.71  |

### ***O<sub>3</sub> Impacts on ClO<sub>4</sub><sup>-</sup> Accumulation***

The soil surface and any Cl<sup>-</sup> in the irrigation water or fertilizer near the surface were exposed directly to O<sub>3</sub>. Soil surface enhancement of ClO<sub>4</sub><sup>-</sup> could occur with increasing O<sub>3</sub> exposure due to soil surface reactions. The average soil surface ClO<sub>4</sub><sup>-</sup> concentration, over all species at the highest O<sub>3</sub> (204 nL L<sup>-1</sup>), was  $7.03 \pm 0.70 \mu\text{g (kg dry wt)}^{-1}$ , significantly greater than the ClO<sub>4</sub><sup>-</sup> concentration of  $3.77 \pm 0.72 \mu\text{g (kg dry wt)}^{-1}$  observed at the lowest O<sub>3</sub>, 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<sub>3</sub>, indicating that placement of the plant and soil in the CSTR, even in the absence of appreciable O<sub>3</sub>, may have caused an increase in ClO<sub>4</sub><sup>-</sup> due to unknown heterogeneous surface reactions.

The ClO<sub>4</sub><sup>-</sup> concentration of surface soil increased linearly with increasing O<sub>3</sub> 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<sub>3</sub> could lead to environmentally significant accumulation of ClO<sub>4</sub><sup>-</sup>. Soil ClO<sub>4</sub><sup>-</sup> does not appear to be attributable to retranslocation from leaves to root exudates. Such exudation of phytoaccumulated ClO<sub>4</sub><sup>-</sup> was not observed into ClO<sub>4</sub><sup>-</sup>-free media in *Populus* (van Aken and Schnoor, 2002).

In contrast to the soil surface, there was no effect of O<sub>3</sub> exposure on foliar content of ClO<sub>4</sub><sup>-</sup> 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<sub>3</sub> exposure and foliar ClO<sub>4</sub><sup>-</sup> accumulation (Fig. 2.7.5).

### ***Stomatal Responses and O<sub>3</sub> Dose***

The use of O<sub>3</sub> concentration as a metric of O<sub>3</sub> exposure has limitations, associated with temporal shifts between stomatal opening and elevated ambient O<sub>3</sub> concentrations (Massman et al., 2000). This is addressed by use of measured or calculated uptake or flux of O<sub>3</sub> into the leaf, (i.e. O<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> exposure in sorghum and bush bean (Fig. 2.7.6C, and D). The decline was proportional at the intermediate O<sub>3</sub> exposure in these species. In soybean and cotton (Table 2.7.8), exposure to 102 nL L<sup>-1</sup> induced a somewhat greater than proportional reduction in  $g_s$ , relative to 204 nL L<sup>-1</sup> O<sub>3</sub>, resulting in a somewhat concave response



curve (not shown). In contrast, maize exhibited a slightly convex response curve. Reduction of mean conductance was between 28% and 83% at the highest O<sub>3</sub>.

With simultaneous measurement of O<sub>3</sub> concentration, we calculated flux of O<sub>3</sub> directly (Section 2.7.1; Grantz et al., 2014). Ozone-induced reduction in stomatal conductance reduced the relative increase in O<sub>3</sub> dose compared with the increase in concentration, at intermediate and high exposures relative to the low [O<sub>3</sub>]. 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 ClO<sub>4</sub><sup>-</sup> and O<sub>3</sub> concentration was found (Fig. 2.7.5) and no relationship was found by use of O<sub>3</sub> 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 ClO<sub>4</sub><sup>-</sup> and O<sub>3</sub> concentration and between ClO<sub>4</sub><sup>-</sup> and O<sub>3</sub> dose, was observed at lower O<sub>3</sub> 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).

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

(\*) significant at  $P < .05$ .

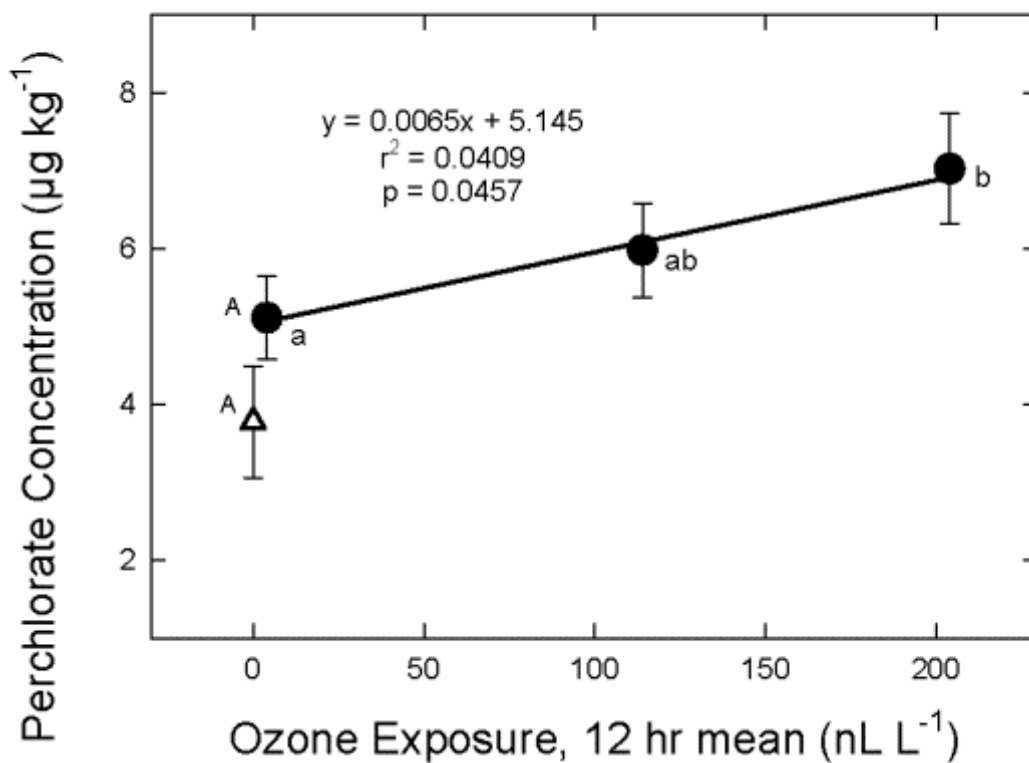


**Figure 2.7.4. Significant relationship between perchlorate concentration ( $\mu\text{g (kg dry wt)}^{-1}$ ) of potting medium exposed in the CSTRs and  $\text{O}_3$  exposure ( $\text{nL L}^{-1}$ ; 12 hr mean;  $r^2 = 0.99$ ;  $P = 0.0714$ ).** The data for unused medium and medium exposed to 4 and  $114 \text{ nL L}^{-1} \text{ O}_3$  are from Grantz et al. (2014). Means followed by different letters are significantly different ( $P < 0.05$ ).

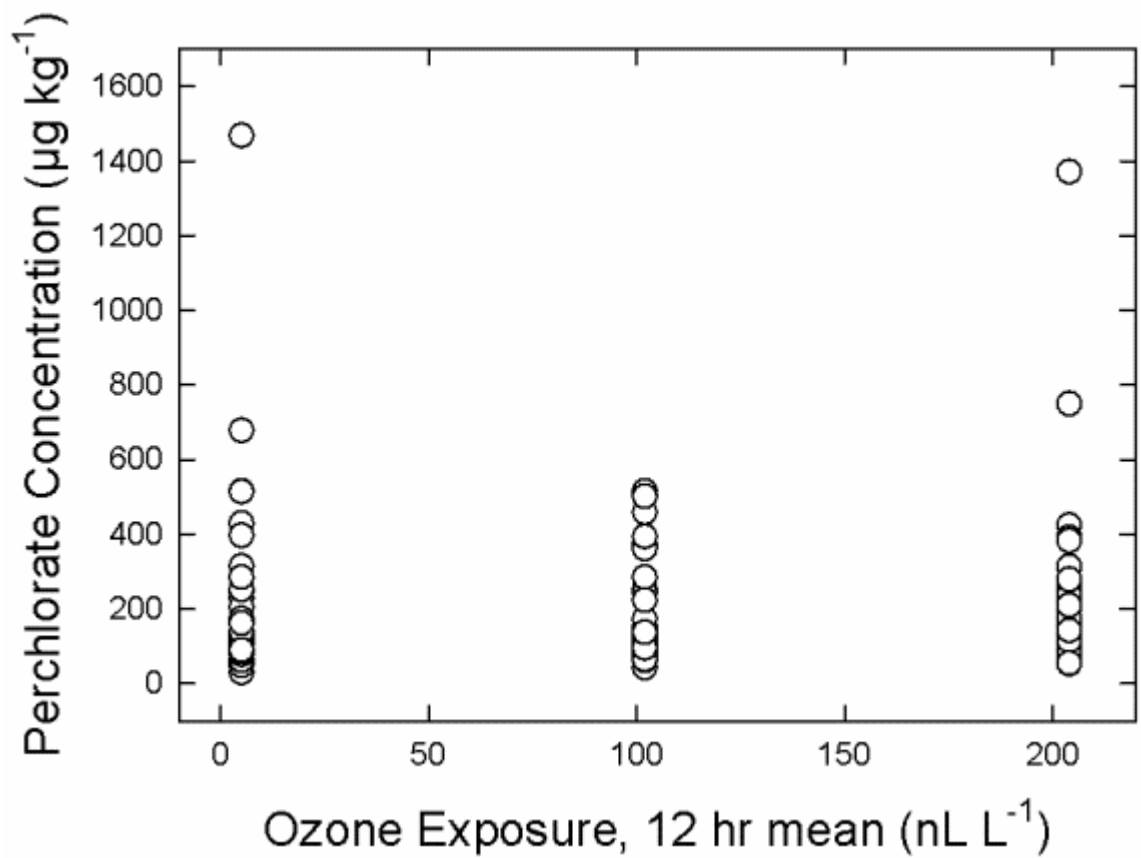


**Figure 2.7.5. No relationship between perchlorate concentration (µg (kg dry wt)<sup>-1</sup>) of young leaves and O<sub>3</sub> exposure.**

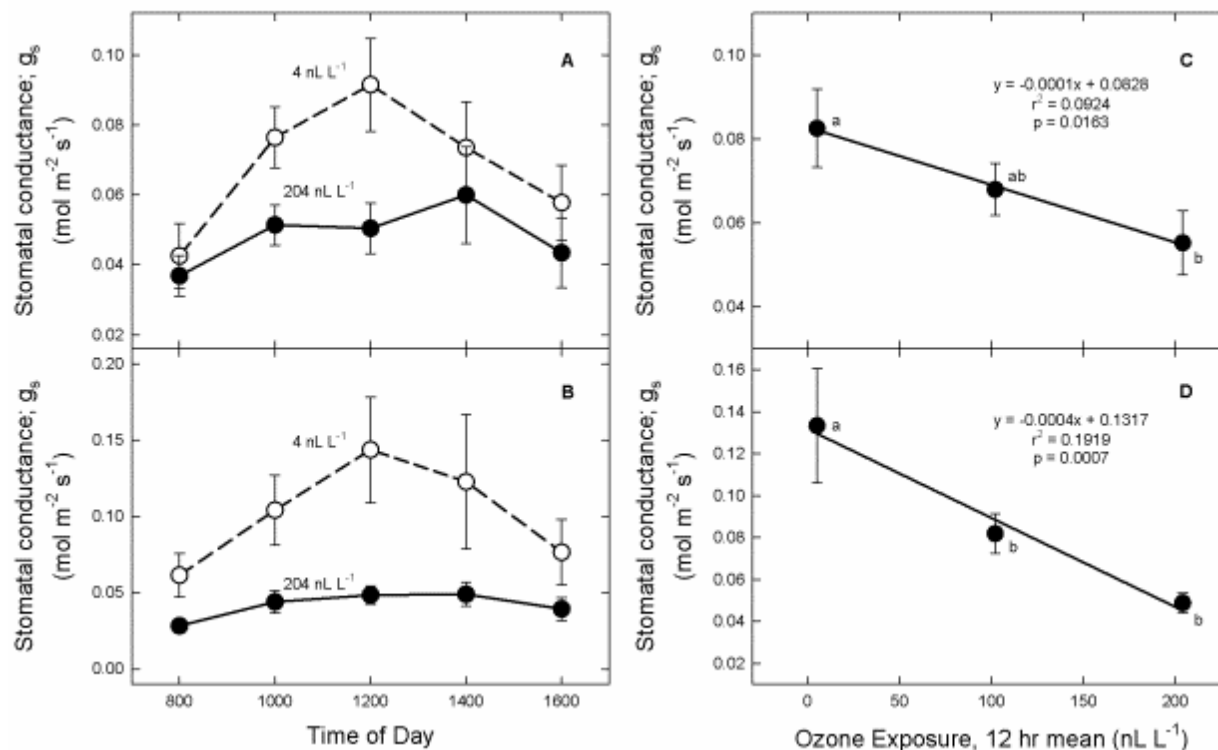


**Figure 2.7.6. Effect of O<sub>3</sub> exposure on stomatal conductance ( $g_s$ ).** (A,B) Diel course of  $g_s$  in (A) sorghum and (B) bush bean at low (open symbols) and high (closed symbols) O<sub>3</sub> exposure. (C,D) Relationship between  $g_s$  of (C) sorghum and (D) bush bean (average of midday values, 12:00 – 14:00), and ozone exposure. Means followed by different letters are significantly different ( $P < 0.05$ ).

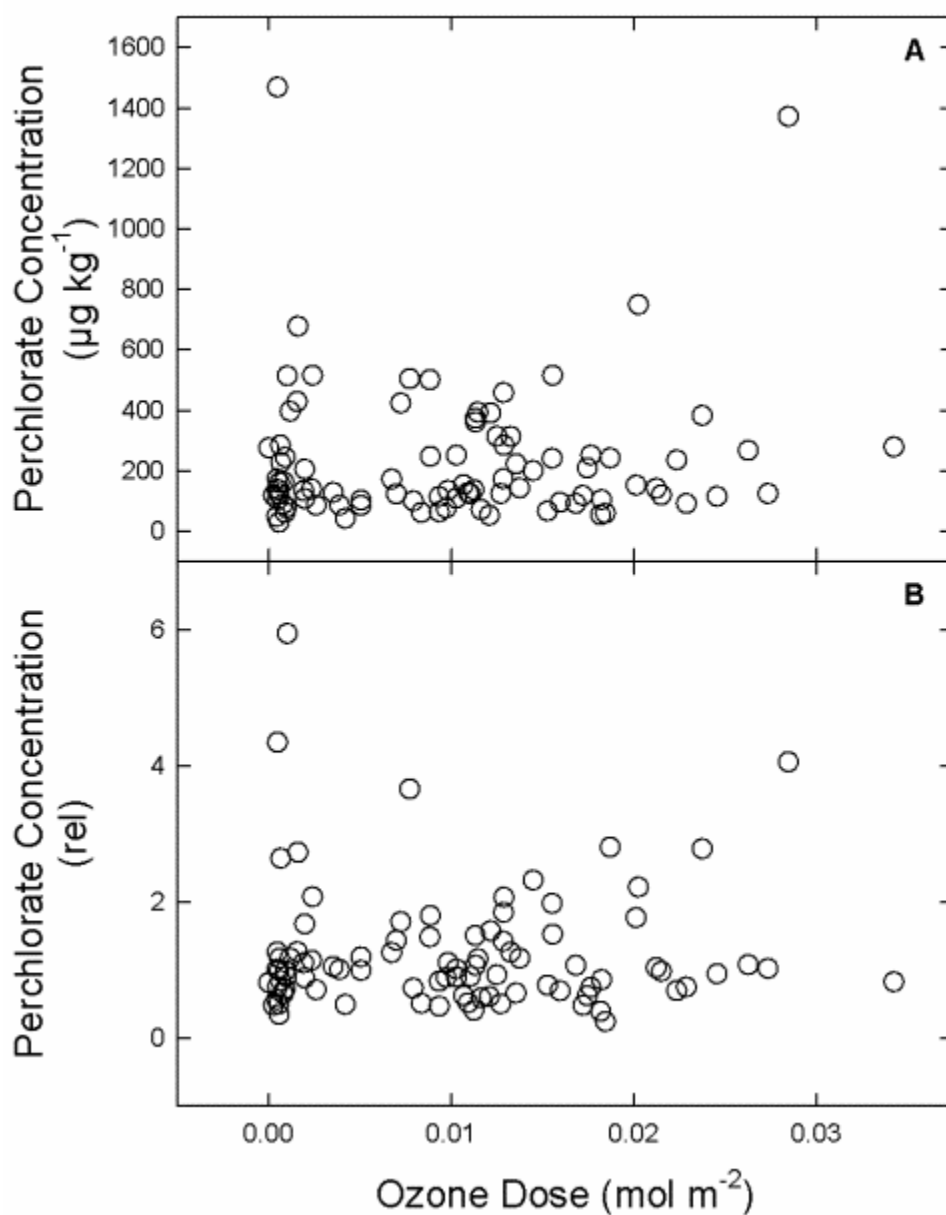


**Figure 2.7.7. No relationship between (A) perchlorate concentration of young leaves ( $\mu\text{g (kg dry wt)}^{-1}$ ) and  $\text{O}_3$  dose; or (B) perchlorate concentration (unitless) normalized by the median concentration over all  $\text{O}_3$  within each species, and  $\text{O}_3$  dose.**

#### 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  $320 \text{ nL L}^{-1}$ , 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 $\text{ClO}_4^-$

### 2.8.1 Background

A process which could affect  $\text{ClO}_4^-$  isotopes is plant uptake; however, its impact on  $\text{ClO}_4^-$  isotopic compositions is not yet clear. It is possible that plants could change the  $\text{ClO}_4^-$  isotopic composition through a variety of mechanisms including: transport carriers in the root; diffusion limitations through the root; reduction of  $\text{ClO}_4^-$  by plant reductases, translocation within the plant, and non-specific exchange of O between  $\text{ClO}_4^-$  and  $\text{H}_2\text{O}$  or other compounds catalyzed by plant compounds.

Uptake of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  accumulation is species and genotype-dependent and most of the  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  entering the plant and the amount of water being transpired, it is inferred that uptake of  $\text{ClO}_4^-$  is at least partially through passive transport, but demonstrated competition for uptake between  $\text{ClO}_4^-$  and other anions like  $\text{NO}_3^-$  and  $\text{HCO}_3^-$  from soil or water indicates that transport is not purely a passive process and that transport proteins may also facilitate transfer of  $\text{ClO}_4^-$  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  $\text{NO}_3^-$  and  $\text{HCO}_3^-$  other anions such as  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{I}^-$ ,  $\text{IO}_3^-$  are not reported to interfere with  $\text{ClO}_4^-$  uptake (Seyfferth et al., 2008 ;Voogt et al., 2010; Marschner, 1995). The influence of  $\text{NO}_3^-$  on  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  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 ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ); (Evans, 2001; Mariotti et al., 1982; Tcherkez et al., 2006; Karsh et al., 2012). By analogy, intracellular processing of  $\text{ClO}_4^-$  within plants could also affect  $\text{ClO}_4^-$  isotope compositions. Isotopic discrimination occurs when  $\text{NO}_3^-$  is reduced and assimilated in plant tissue by the nitrate reductase enzyme, causing an increase in both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of the residual  $\text{NO}_3^-$  (Andraski et al., 2014). Although processes by which  $\text{ClO}_4^-$  might be metabolized within plants are unknown, considering that  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  might share the same uptake mechanism, it is possible that  $\text{ClO}_4^-$



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  $\text{ClO}_4^-$  reduction in plants. Such processes in plants could affect the isotopic characteristics of  $\text{ClO}_4^-$  returned to soils and groundwaters if residual  $\text{ClO}_4^-$  is released back into the environment, which appears likely (Van Aken et al., 2002; Nzungu et al., 2004; Susalara et al., 2000; Laursen et al., 2013). Improved understanding of such processes could be useful for interpreting origins of environmental  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ . The relative abundance and isotopic composition of  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  extracted from plants and to determine whether isotopic exchange or fractionation (by transport or degradation) might alter the isotopic composition of  $\text{ClO}_4^-$  accumulating in plants. This was accomplished by comparing the stable isotopic compositions of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  might not be a reliable indicator of  $\text{ClO}_4^-$  stability unless extractable  $\text{NO}_3^-$  exhibited substantial fractionation effects. We also evaluated  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in the food chain, we analyzed commercially grown spinach and snap bean plants exposed only to background concentrations of  $\text{ClO}_4^-$ .

## 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  $\text{ClO}_4^-$  or isotopically labeled  $\text{ClO}_4^-$ . 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  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$  uptake. Isotopic analyses were performed on  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  (0.01, 2, and 10 mg/L) along with  $\text{NO}_3^-$  and other ions. Two control treatments were also evaluated. One control contained no plants and 1 mg/L  $\text{ClO}_4^-$  and the other contained plants and had no  $\text{ClO}_4^-$ . The  $\text{ClO}_4^-$  for the 0.01, 1, and 10 mg/L exposures was supplied from a 98% sodium perchlorate ( $\text{NaClO}_4$ ) lab reagent (ACROS Organics) while the  $\text{ClO}_4^-$  used in the 2 mg/L exposure was supplied from an isotopically labeled  $\text{KClO}_4$  reagent (USGS38) distributed by the USGS (Reston, VA). All treatments were evaluated to determine the amount of  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  lost from solution over time and the amount of  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  accumulated in plant tissue, while a subset of treatments (1 mg/L no plants control, 2 mg/L  $\text{ClO}_4^-$ , and 10 mg/L  $\text{ClO}_4^-$ ) were evaluated for stable isotopes.

**Table 2.8.1 Summary of initial experimental conditions for  $\text{ClO}_4^-$  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.

| Parameter                 | Experiment 1                       |         |           |           | Experiment 2                       |         |          |          |
|---------------------------|------------------------------------|---------|-----------|-----------|------------------------------------|---------|----------|----------|
|                           | $\text{ClO}_4^-$ Treatments (mg/L) |         |           |           | $\text{ClO}_4^-$ Treatments (mg/L) |         |          |          |
|                           | 0                                  | 1       | 2         | 10        | 0                                  | 1       | 0.01     | 10       |
| $\text{Cl}^-$ (mg/L)      | 1.2 (0)                            | 1.0 (0) | 1.3 (0.4) | 1.7 (0.3) | 103 (0)                            | 105 (0) | 108 (1)  | 108 (3)  |
| $\text{NO}_3^-$ -N (mg/L) | 274 (0)                            | 268 (0) | 274 (3)   | 264 (13)  | 237 (0)                            | 240 (0) | 237 (2)  | 235 (4)  |
| $\text{PO}_4^{3-}$ (mg/L) | 48 (0)                             | 46 (0)  | 72 (17)   | 59 (23)   | 72 (0)                             | 74 (0)  | 76 (5)   | 70 (1)   |
| $\text{SO}_4^{2-}$ (mg/L) | 117 (0)                            | 110 (0) | 116 (2)   | 113 (9)   | 130 (0)                            | 137 (0) | 144 (12) | 142 (15) |
| # tubs (replicates)       | 1                                  | 1       | 4         | 2         | 1                                  | 1       | 2        | 2        |
| Plants per tub            | 6                                  | 0       | 6         | 6         | 4                                  | 0       | 4        | 4        |

Anion concentrations reported are the means of the replicates for each  $\text{ClO}_4^-$  treatment. Standard errors of the means are indicated in parenthesis (e.g. Mean ( $\pm$ 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  $\times$  36.87cm  $\times$  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  $\text{HNO}_3$ . 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  $\text{ClO}_4^-$  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

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  $\text{ClO}_4^-$  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 ( $\text{Al}_2\text{O}_3$ ) 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  $\mu\text{m}$ ) syringe filter and a Dionex RP cartridge. Cleaned samples from each treatment were analyzed for  $\text{ClO}_4^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ -N,  $\text{SO}_4^{2-}$ , and  $\text{PO}_4^{3-}$  and a small volume was set aside and preserved for  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  isotopic analysis. The pooled leaf extracts for each treatment as well as the remaining nutrient solutions, excluding the 0.01 mg/L and no  $\text{ClO}_4^-$  control plant extraction and growth solutions, were pumped through Purolite A-530E resin columns to capture the  $\text{ClO}_4^-$  (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  $\text{ClO}_4^-$ . 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  $\text{ClO}_4^-$  ( $\sim 5 \mu\text{g/L}$ ). In this case, a Purolite® A-530E resin filter was used to reduce  $\text{ClO}_4^-$  levels. Irrigation water at the NY site was found to contain  $\sim 0.3$  ppb  $\text{ClO}_4^-$ , 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  $60^\circ\text{C}$ . Dried leaf tissue was stored in large plastic bags to exclude moisture and prevent contamination and then shipped to Texas Tech University for  $\text{ClO}_4^-$  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  $0.45 \mu\text{m}$  filter and Purolite® A-530E resin columns. The resin columns were stored at  $4^\circ\text{C}$  until processed for  $\text{ClO}_4^-$  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.

Solutes 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  $0.45 \mu\text{m}$  filter and Purolite A-530E resin column. The extraction column was preserved with HCl at a pH  $\leq 2$ .

#### 2.8.2.5 Sample Analyses

Concentrations of  $\text{ClO}_4^-$  and major anions ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ) 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  $0.05 \mu\text{g/L}$  ( $\text{ClO}_4^-$ ) and ion chromatography (EPA Method 300.0) with a reporting limit of  $0.5 \text{ mg/L}$  (other anions) as previously described in this report. The  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  was shipped to the USGS in Reston Virginia for analysis of  $\delta^{18}\text{O}$ ,  $\delta^{17}\text{O}$ , and  $\Delta^{17}\text{O}$  of  $\text{O}_2$  produced by decomposition of  $\text{ClO}_4^-$  and the  $\text{Cl}^-$  residue from the decomposed  $\text{ClO}_4^-$  was analyzed for  $\delta^{37}\text{Cl}$  at the University of Illinois at Chicago (Hatzinger et al., 2011). The  $\text{NO}_3^-$  stable isotope ratios ( $\delta^{18}\text{O}$ ,  $\delta^{15}\text{N}$ ) 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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  were from Böhlke et al. (2009) and Böhlke et al. (2003), respectively. Selected samples were analyzed for  $\text{NO}_2^-$  concentration and isotopic composition, as  $\text{NO}_2^-$  can interfere with  $\text{NO}_3^-$  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  $\text{ClO}_4^-$ , 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.

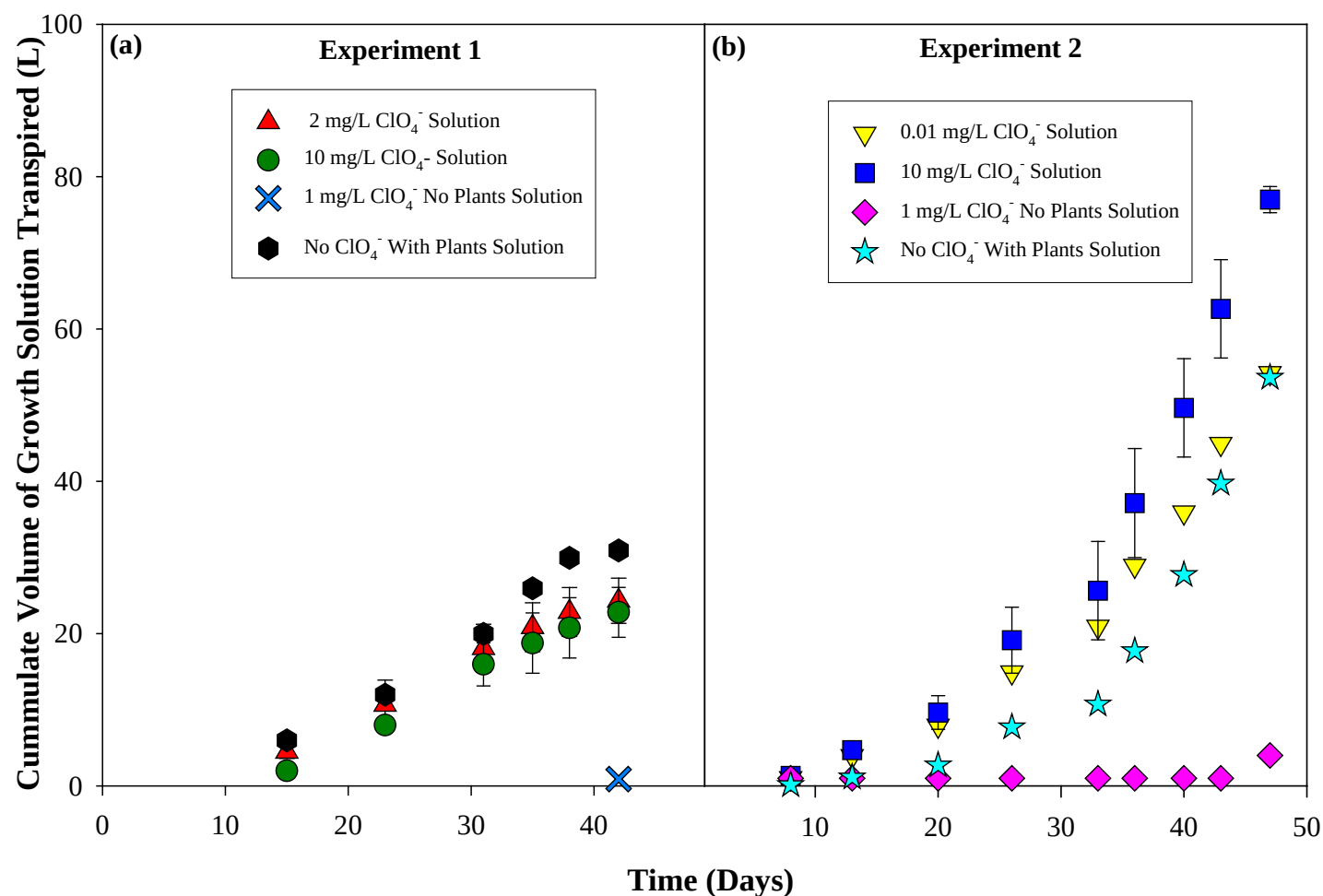


**Figure 2.8.1. Cumulative volume of growth solution uptaken by pooled replicate treatments in both experiments.**

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  $\text{ClO}_4^-$  treatment.

As DDI water was added to replace transpired water,  $\text{ClO}_4^-$  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),  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  from solution (Figure 2.8.2a and 2.8.2d). The fractional mass loss of  $\text{ClO}_4^-$  from solution was independent of initial  $\text{ClO}_4^-$  concentration, but there was a larger overall decrease in  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  solution mass in treatments with no plants in experiment 1, but not experiment 2. Linear correlations that were independent of initial  $\text{ClO}_4^-$  treatment concentration ( $r^2 = 0.82$  and  $0.95$  for  $2 \text{ mg/L}$  and  $10 \text{ mg/L}$   $\text{ClO}_4^-$ , respectively,  $P < 0.05$ ) were observed between the amount of solution transpired and the fraction of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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.



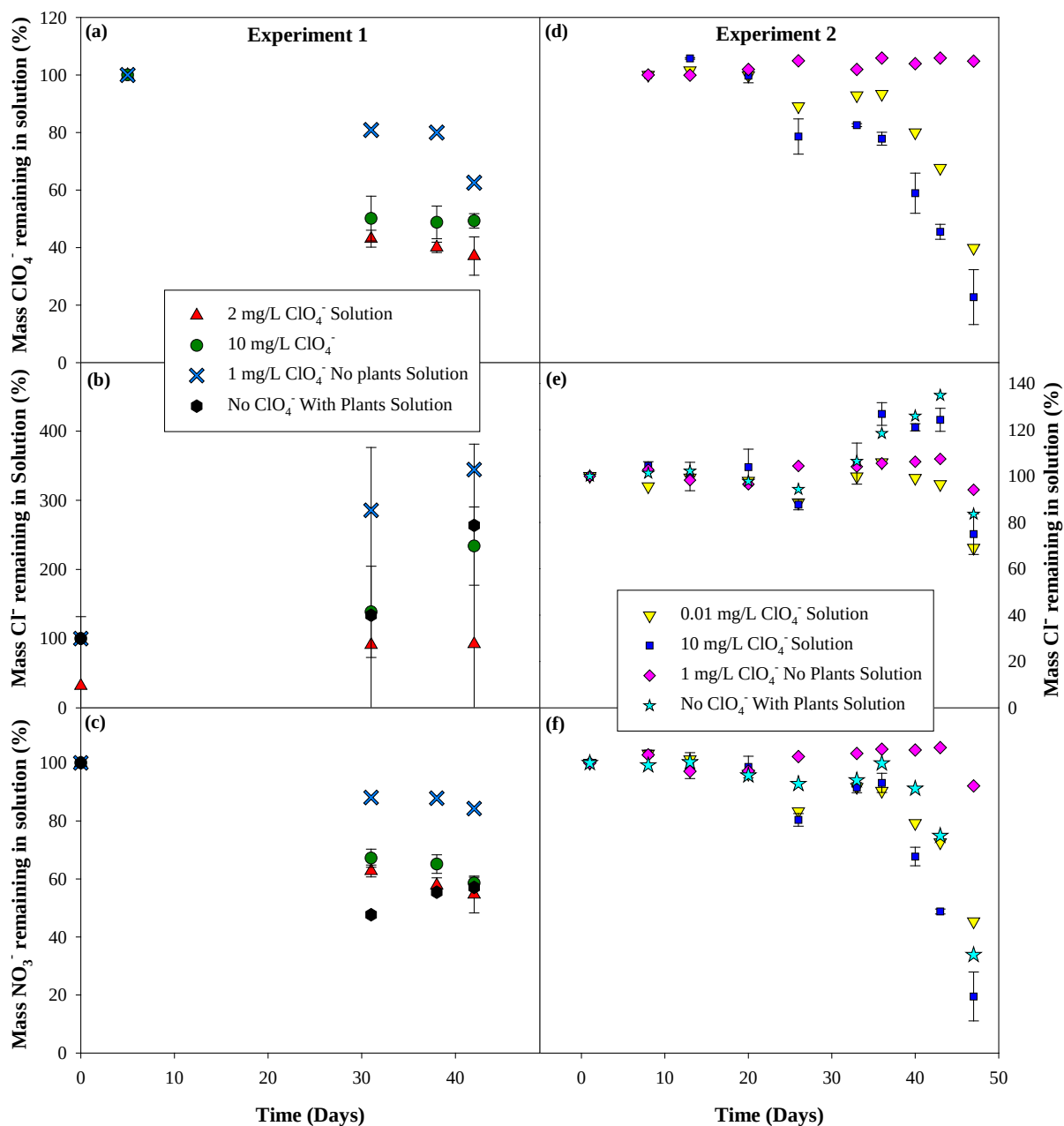


**Figure 2.8.2. Fraction of  $\text{ClO}_4^-$ ,  $\text{Cl}^-$ , and  $\text{NO}_3^-$  mass remaining in nutrient solutions at the time of harvest.** Values reported are the average of pooled replicates in each treatment. Experiment 1 data is represented in graph panels (a), (b), and (c) while experiment 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  $\text{ClO}_4^-$  treatment.

Like  $\text{ClO}_4^-$ , the  $\text{NO}_3^-$  concentrations in solutions of treatments with plants decreased gradually over time in a non-linear fashion and there was smaller  $\text{NO}_3^-$  loss (< 20% of total  $\text{NO}_3^-$ ) in the no plant treatments (Figure 2.8.2c and 2.8.2f and Table 2.8.2). The  $\text{NO}_3^-$  fractional mass loss was linearly correlated ( $P < 0.05$ ) with the amount of solution transpired regardless of initial  $\text{ClO}_4^-$  treatment concentration ( $r^2 = 0.76, 0.83, 0.86$  for 0, 0.01, and 10 mg/L  $\text{ClO}_4^-$ , respectively) (Figure 2.8.3c). Both  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  had similar uptake rates as % mass/L transpired ( $-0.8683 \pm 0.15$  to  $-0.9912 \pm 0.21$ ) and ( $-0.97 \pm 0.08$  to  $-1.02 \pm 0.17$ ), respectively, supporting a similar uptake mechanism, as also suggested previously by other studies (Seyfferth et al., 2008; Marschner, 1995).

In contrast to  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ ,  $\text{Cl}^-$  mass in solution remained relatively constant (~ 80-120%) throughout the experiment regardless of initial  $\text{ClO}_4^-$  treatment concentration and presence or absence of plants (Figure 2.8.2e). There was no correlation between mass of  $\text{Cl}^-$  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  $\text{Cl}^-$  clearly was accumulated in plants, the amount taken up was small compared to the amount in solution (high –  $\text{Cl}^-$  in experiment 2) or small compared to analytical uncertainty (low –  $\text{Cl}^-$  in experiment 1).

Table 2.8.2. Average  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$  and total-N fractional mass distribution in growth solution and snap bean plant tissue.

| Parameter                                                           | Experiment 1: $\text{ClO}_4^-$ Treatments (mg/L) |           |            |            | Experiment 2: $\text{ClO}_4^-$ Treatments (mg/L) |           |            |            |
|---------------------------------------------------------------------|--------------------------------------------------|-----------|------------|------------|--------------------------------------------------|-----------|------------|------------|
|                                                                     | 0                                                | 1         | 2          | 10         | 0                                                | 0.01      | 1          | 10         |
| % $\text{ClO}_4^-$ mass of initial total $\text{ClO}_4^-$ mass (SD) |                                                  |           |            |            |                                                  |           |            |            |
| Final Solution                                                      | N/A                                              | 62.5(0.0) | 37.1(6.7)  | 49.3(2.5)  | N/A                                              | 39.8(0.0) | 104.8(0.0) | 22.8(9.6)  |
| Leaves                                                              | N/A                                              | N/A       | 16.3(1.7)  | 17.9(7.2)  | N/A                                              | 41.4(0.0) | N/A        | 53.7(6.5)  |
| Bean Pods                                                           | N/A                                              | N/A       | 4.1(0.5)   | 4.9(0.4)   | N/A                                              | 2.2(0.0)  | N/A        | 7.3(0.6)   |
| Stems                                                               | N/A                                              | N/A       | 0.26(0.04) | 0.29(0.07) | N/A                                              | 1.3(0.0)  | N/A        | 0.98(0.18) |
| Roots                                                               | N/A                                              | N/A       | 0.22(0.09) | 0.24(0.09) | N/A                                              | 0.9(0.0)  | N/A        | 0.59(0.02) |
| Rockwool                                                            | N/A                                              | N/A       | 0.11(0.01) | 0.15(0.00) | N/A                                              | NM        | N/A        | NM         |
| Unaccounted                                                         | N/A                                              | 37.5(0.0) | 42.0(7.3)  | 27.3(4.4)  | N/A                                              | 14.3(0.0) | -4.8(0.0)  | 19.8(3.4)  |
| % $\text{NO}_3^-$ mass of initial total $\text{NO}_3^-$ mass (SD)   |                                                  |           |            |            |                                                  |           |            |            |
| Final Solution                                                      | 57.1(0.0)                                        | 84.3(0.0) | 54.7(6.4)  | 58.6(2.1)  | 33.8(0.0)                                        | 45.3(0.0) | 92.0(0.0)  | 19.5(8.4)  |
| Leaves                                                              | 0.45(0.0)                                        | N/A       | 0.49(0.08) | 0.29(0.14) | 4.0(0.0)                                         | 1.4(0.0)  | N/A        | 1.9(1.7)   |
| Bean Pods                                                           | 0.45(0.0)                                        | N/A       | 0.41(0.03) | 0.36(0.01) | 0.26(0.0)                                        | 0.6(0.0)  | N/A        | 1.3(0.7)   |
| Stems                                                               | 0.52(0.0)                                        | N/A       | 0.61(0.15) | 0.4(0.0)   | 1.4(0.0)                                         | 1.2(0.0)  | N/A        | 1.7(0.2)   |
| Roots                                                               | 0.31(0.0)                                        | N/A       | 0.33(0.10) | 0.27(0.10) | 4.6(0.0)                                         | 2.4(0.0)  | N/A        | 3.3(0.3)   |
| Rockwool                                                            | NM                                               | NM        | NM         | NM         | NM                                               | NM        | NM         | NM         |
| Unaccounted                                                         | 41.2(0.0)                                        | 15.7(0.0) | 43.5(6.4)  | 40.1(1.8)  | 55.8(0.0)                                        | 49.1(0.0) | 8.0(0.0)   | 72.3(6.9)  |
| % $\text{Cl}^-$ mass of initial total $\text{Cl}^-$ mass (SD)       |                                                  |           |            |            |                                                  |           |            |            |
| Final Solution                                                      | N/A                                              | N/A       | N/A        | N/A        | 78.2(0.0)                                        | 77.3(0.0) | 101.0(0.0) | 73.0(13.0) |
| Leaves                                                              | N/A                                              | N/A       | N/A        | N/A        | 2.0(0.0)                                         | 5.8(0.0)  | N/A        | 3.9(1.3)   |
| Bean Pods                                                           | N/A                                              | N/A       | N/A        | N/A        | 0.85(0.0)                                        | 2.0(0.0)  | N/A        | 3.0(1.8)   |
| Stems                                                               | N/A                                              | N/A       | N/A        | N/A        | 3.6(0.0)                                         | 4.7(0.0)  | N/A        | 4.6(2.8)   |
| Roots                                                               | N/A                                              | N/A       | N/A        | N/A        | 2.0(0.0)                                         | 1.3(0.0)  | N/A        | 1.6(1.0)   |
| Rockwool                                                            | N/A                                              | N/A       | N/A        | N/A        | NM                                               | NM        | N/A        | NM         |
| Unaccounted                                                         | N/A                                              | N/A       | N/A        | N/A        | 13.2(0.0)                                        | 8.6(0.0)  | -1.0(0)    | 13.6(9.6)  |
| % total-N mass of Initial total-N mass (SD)                         |                                                  |           |            |            |                                                  |           |            |            |
| Final Solution                                                      | 57.1(0.0)                                        | 84.3(0.0) | 54.7(6.4)  | 58.6(2.1)  | NM                                               | NM        | NM         | NM         |
| Leaves                                                              | 12.8(0.0)                                        | N/A       | 10.8(1.6)  | 13.8(3.9)  | NM                                               | NM        | NM         | NM         |
| Bean Pods                                                           | 14.3(0.0)                                        | N/A       | 13.8(1.0)  | 12.4(1.6)  | NM                                               | NM        | NM         | NM         |
| Stems                                                               | 3.8(0.0)                                         | N/A       | 3.9(0.9)   | 3.2(0.9)   | NM                                               | NM        | NM         | NM         |
| Roots                                                               | 2.7(0.0)                                         | N/A       | 3.1(1.6)   | 2.7(1.0)   | NM                                               | NM        | NM         | NM         |
| Rockwool                                                            | NM                                               | NM        | NM         | NM         | NM                                               | NM        | NM         | NM         |
| Unaccounted                                                         | 9.4(0.0)                                         | 15.7(0.0) | 14.3(6.0)  | 9.3(5.3)   | NM                                               | NM        | NM         | NM         |

NM = Not Measured

N/A = Not Applicable

(SD) = Standard deviation

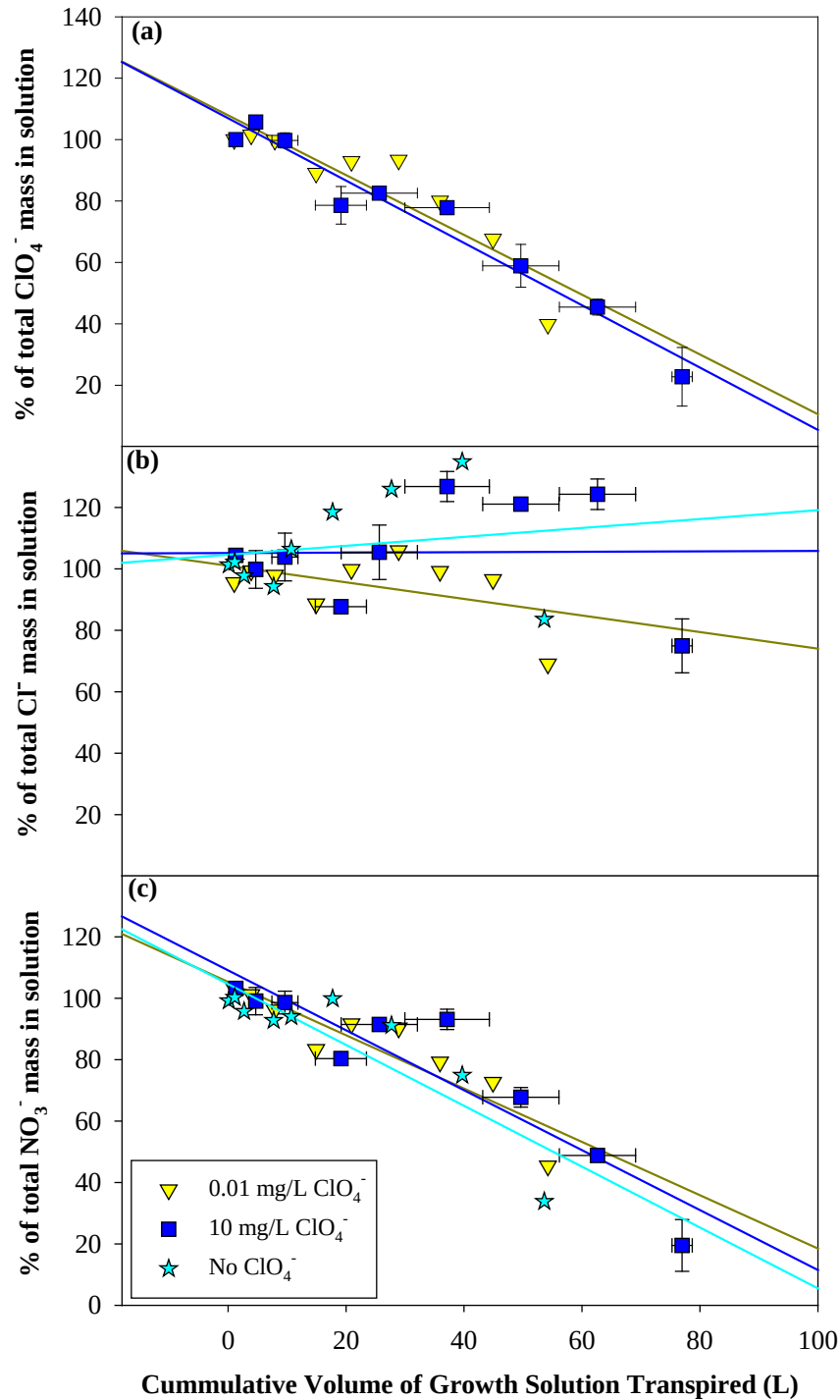


**Figure 2.8.3. Relationship between fraction of total (a)  $\text{ClO}_4^-$ , (b)  $\text{Cl}^-$ , and (c)  $\text{NO}_3^-$  mass lost from solution and cumulative volume of solution transpired over the course of experiment 2.** The grayish yellow regression lines correspond to the 0.01 mg/L  $\text{ClO}_4^-$  treatment, the dark blue regression lines correspond to the 10 mg/L  $\text{ClO}_4^-$  treatment, and the aqua blue regression lines correspond to the no  $\text{ClO}_4^-$  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  $\text{H}_2\text{O}$  by transpiration in experiment 2. To facilitate comparisons, leaf bioconcentration factors (BCF) were calculated using Equation 1.

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

Combined results of both experiments yielded  $\text{ClO}_4^-$  BCF values of  $66 \pm 0$ ,  $64 \pm 3$ , and  $63 \pm 11$  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  $27.3 \pm 4.4$  to  $42.0 \pm 7.3\%$  and  $14.3 \pm 0.0$  to  $19.8 \pm 3.4\%$  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 ( $1.56 \pm 0.76$ ,  $0.49 \pm 0$ ,  $1.38 \pm 0.11$ , and  $1.40 \pm 0.63$  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 ( $\sim 15.7 \pm 0$  to  $43.5 \pm 6.4\%$  and  $8.0 \pm 0$  to  $72.3 \pm 6.9\%$  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  $\text{ClO}_4^-$  as opposed to  $\text{Cl}^-$ . Plants did not accumulate much  $\text{Cl}^-$ , but of the  $\text{Cl}^-$  in the plant,  $\text{Cl}^-$  percent mass was distributed similarly between leaves and stems (24-43%) and between bean pods and roots (10-24%). The fraction of  $\text{Cl}^-$  mass recovered in the snap bean plant and the  $\text{Cl}^-$  mass not accounted for were comparable ( $\sim 9$ -15%) (Table 2.8.2).

### ***Perchlorate Isotopes***

The role of plant uptake on  $\text{ClO}_4^-$  isotopic composition was determined by evaluating the isotopic composition of solution and plant leaf  $\text{ClO}_4^-$ . To minimize ambiguity in the results, two sources of  $\text{ClO}_4^-$  were used in the experiments: a normal synthetic  $\text{NaClO}_4$  lab reagent and a  $\text{KClO}_4$  salt that was depleted in  $^{37}\text{Cl}$  and enriched in  $^{18}\text{O}$  and  $^{17}\text{O}$  (USGS38, with large positive  $\Delta^{17}\text{O}$ ). With few exceptions, leaf and solution  $\text{ClO}_4^-$  isotopic composition ( $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ ,  $\Delta^{17}\text{O}$ ) were not consistently different from each other in both experiments using both  $\text{ClO}_4^-$  sources ( $n = 4$  pairs) (Figure 2.8.4). For most experiments using isotopically anomalous  $\text{ClO}_4^-$  (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  $\text{ClO}_4^-$  replicate treatments in experiment 1 had anomalously high  $\delta^{18}\text{O}$  and relatively high  $\delta^{37}\text{Cl}$  in leaf tissue, but not difference in  $\Delta^{17}\text{O}$  (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  $\text{NO}_3^-$  extracted from that sample was not anomalous. For experiments with normal reagent  $\text{ClO}_4^-$  (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  $\text{ClO}_4^-$  extraction, purification, and isotopic analysis, these data indicate isotopic fractionation or exchange attributable to plant  $\text{ClO}_4^-$  uptake, plant  $\text{ClO}_4^-$  transformation, microbial  $\text{ClO}_4^-$  transformation, or isotopic exchange between  $\text{ClO}_4^-$  and other constituents during the experiments was generally insignificant.

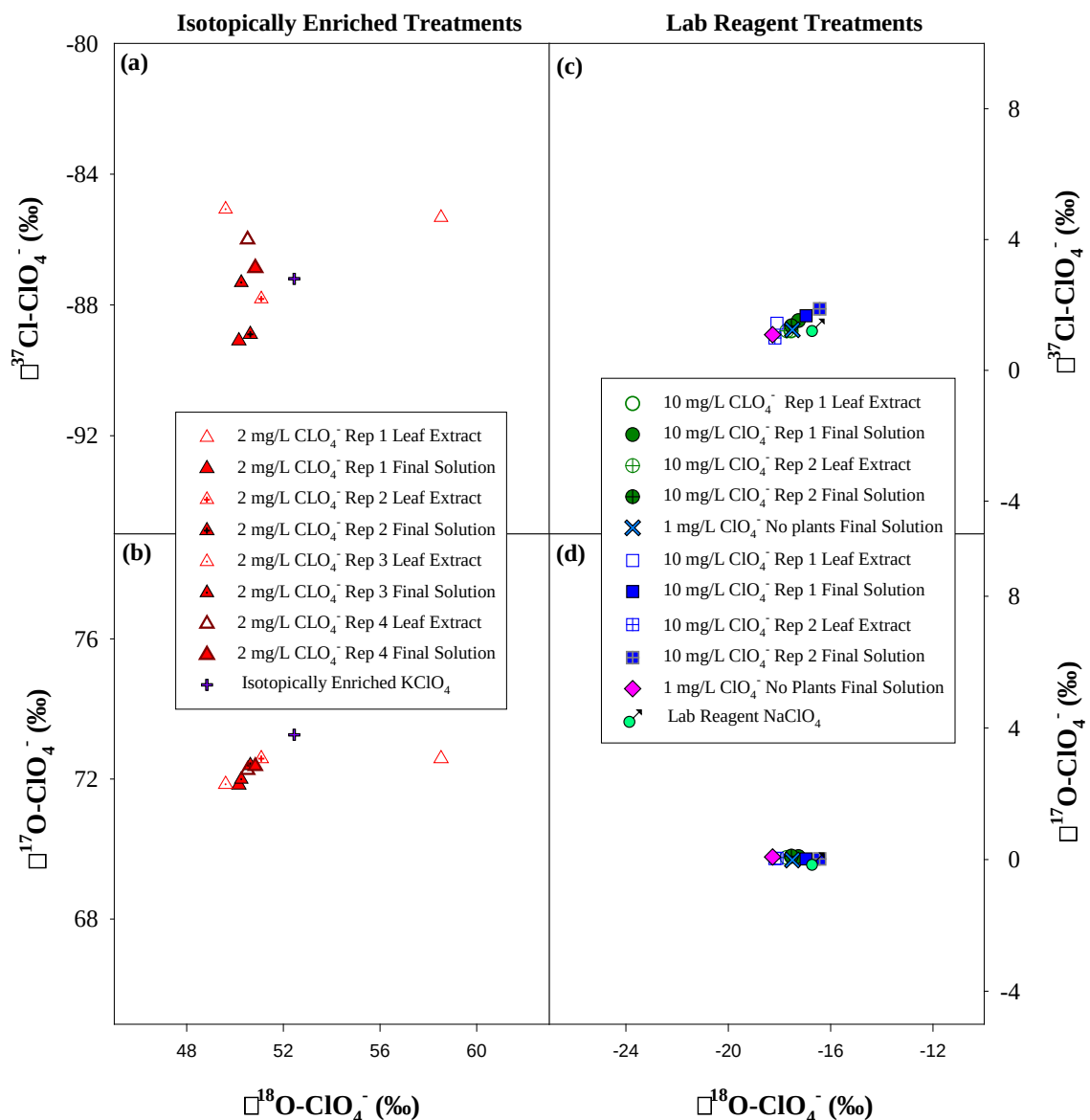


**Figure 2.8.4. Stable  $\text{ClO}_4^-$  isotopic composition of nutrient solutions and leaf extracts of replicate treatments in experiment 1 and 2.** Experiment 1 is represented by the red triangle, green circle, and light blue cross symbols, while experiment 2 is represented by the dark blue square and pink diamond symbols.  $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$  (O2-DI), and  $\Delta^{17}\text{O}$  values are referenced to 0 for SMOC, VSMOW, and VSMOW, respectively.

### ***Nitrate Isotopes***

Hydroponic solution  $\text{NO}_3^-$   $\delta^{15}\text{N}$  values appear to have increased slightly ( $< 2\text{‰}$ ) 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,  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  increases,  $\delta^{18}\text{O}$  also increased significantly, whereas  $\delta^{18}\text{O}$  variations in other treatments were within normal analytical uncertainties. Apparent isotope fractionation factors ( $\epsilon$ ) derived from residual  $\text{NO}_3^-$  in solution were of the order of  $-0.6$  to  $-1.5\text{‰}$  for  $\epsilon(15/14)$  and  $-0.4$  to  $-3.1\text{‰}$  for  $\epsilon(18/16)$  (Figure 2.8.6). Correlated increases in  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in late stages of two treatments could indicate minor fractionation by plant uptake or  $\text{NO}_3^-$  reduction, but the apparent effects in solution were much smaller than those attributable to assimilation within the plants (Figure 2.8.5). Apparent  $\epsilon^{15}\text{N}/\epsilon^{18}\text{O}$  values for  $\text{NO}_3^-$  in solution of around  $0.5$ - $0.6$  (Figure 2.8.6f) were lower than most reported values for  $\text{NO}_3^-$  reduction ( $\epsilon^{15}\text{N}/\epsilon^{18}\text{O} \approx 1$ ) and possibly more like those reported for  $\text{NO}_3^-$  transport from solution into plants (Needoba et al., 2003, 2004; Granger et al., 2010) though overall uncertainties are large.



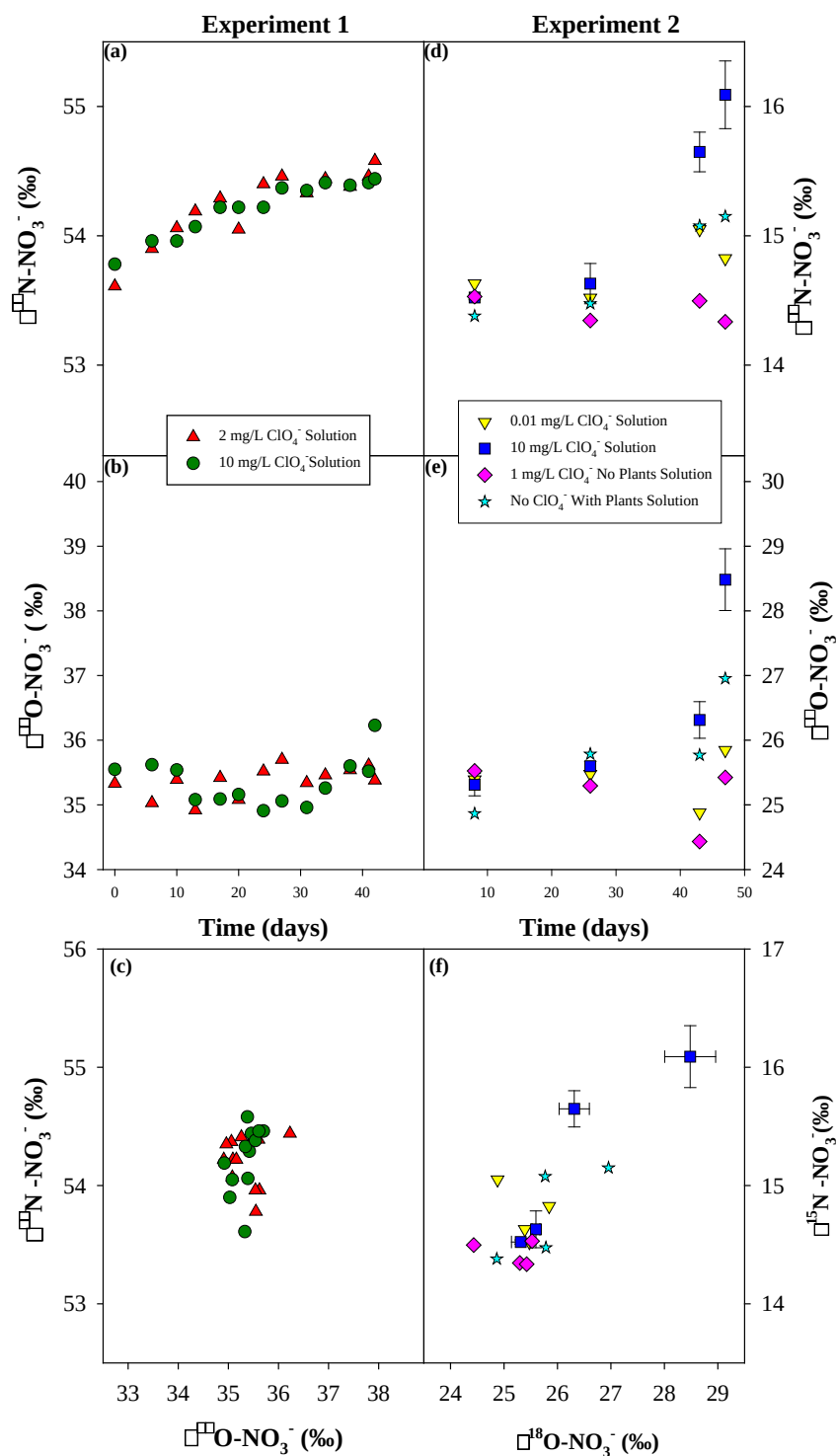


**Figure 2.8.5. Stable isotopic composition of  $\text{NO}_3^-$  in nutrient solutions as a function of time for experiment 1 (a, b) and experiment 2 (d, e).** Panels (c) and (f) show the corresponding relation between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ . Initial  $\text{NO}_3^-$  isotopic compositions differed between the experiments. Only a subset of solution samples was analyzed in experiment 2.

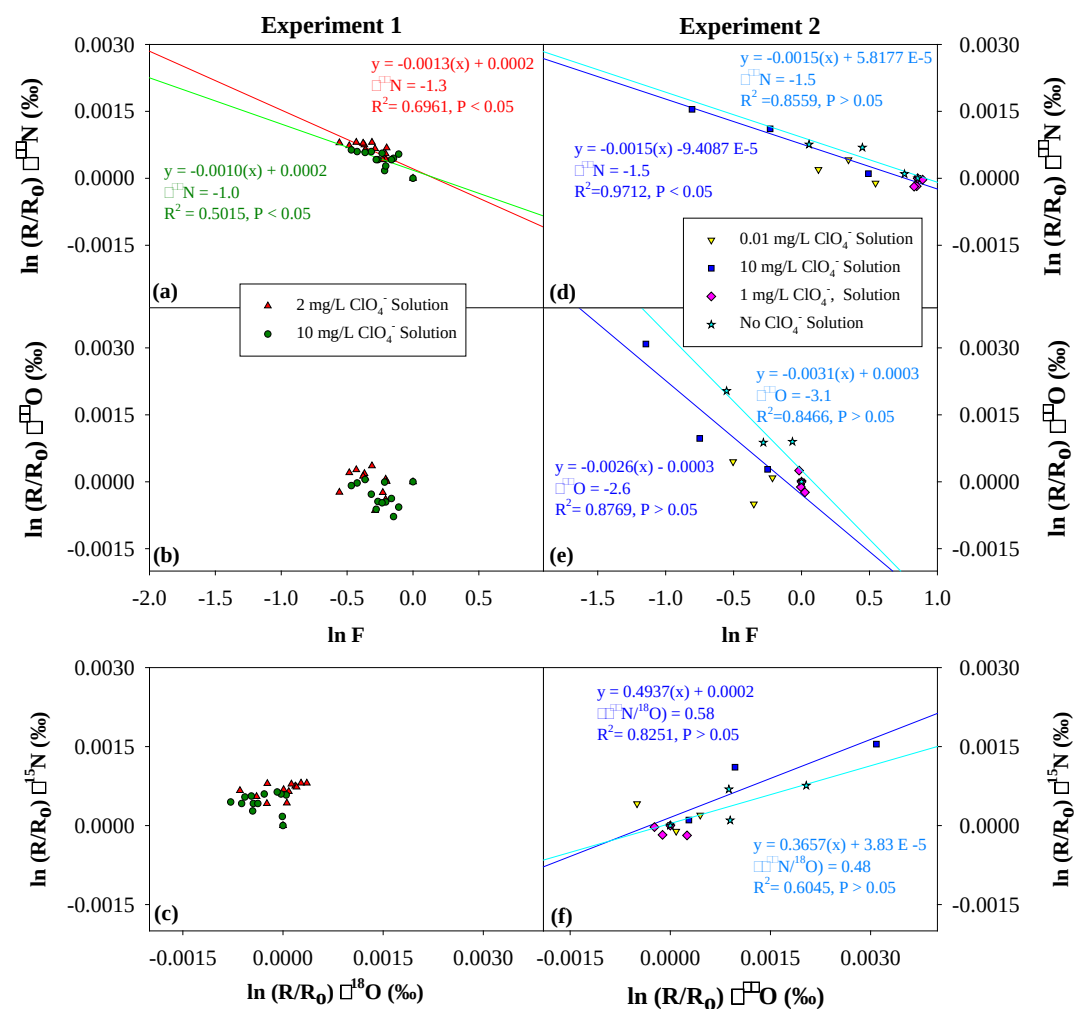


**Figure 2.8.6** Rayleigh plots of the change in the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  in nutrient solutions of experiment 1(a, b, c) and experiment 2 (d, e, f). The colors of the regression lines and text are the same as those of the symbols for the treatments they represent (e.g. red = 2 mg/L  $\text{ClO}_4^-$ ).

$\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of  $\text{NO}_3^-$  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  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of leaves and growth solutions with ratios ( $\Delta^{15}\text{N}/\Delta^{18}\text{O}$ ) of 1.07 (experiment 1) and 1.04 (experiment 2), consistent with reported effects of  $\text{NO}_3^-$  reduction during assimilation by plants (Granger et al., 2004; Trull et al., 2008, DiFiore et al., 2009). This is supported by  $\delta^{15}\text{N}$  measurements of plants from experiment 1. Total N- $\delta^{15}\text{N}$  varied by around 3.4 ‰ in different plant compartments (i.e. roots, stems, leaves, and bean pods). Highest  $\delta^{15}\text{N}$  values were in stems ( $\pm 34 \pm 3$  ‰) and lowest values were in roots ( $\pm 29 \pm 4$  ‰). The weighted composite mean  $\delta^{15}\text{N}$  value for whole plants in experiment 1 was  $+31 \pm 4$  ‰. 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,  $\delta^{15}\text{N}$  values were  $\sim +54$  ‰ for solution  $\text{NO}_3^-$ ,  $\sim +31$  ‰ for final plant tissue, and  $\sim +72$  ‰ for final residual  $\text{NO}_3^-$  extracted from leaves. These results are quantitatively consistent with isotope effects dominated by internal  $\text{NO}_3^-$  reduction during assimilation. Plant total-N isotope data are not available for experiment 2, but isotopic contrasts between solution  $\text{NO}_3^-$  and leaf  $\text{NO}_3^-$  were similar to those in experiment 1, even though the  $\text{NO}_3^-$  reagents used in the experiments had different initial isotopic compositions (Figure 2.8.7).

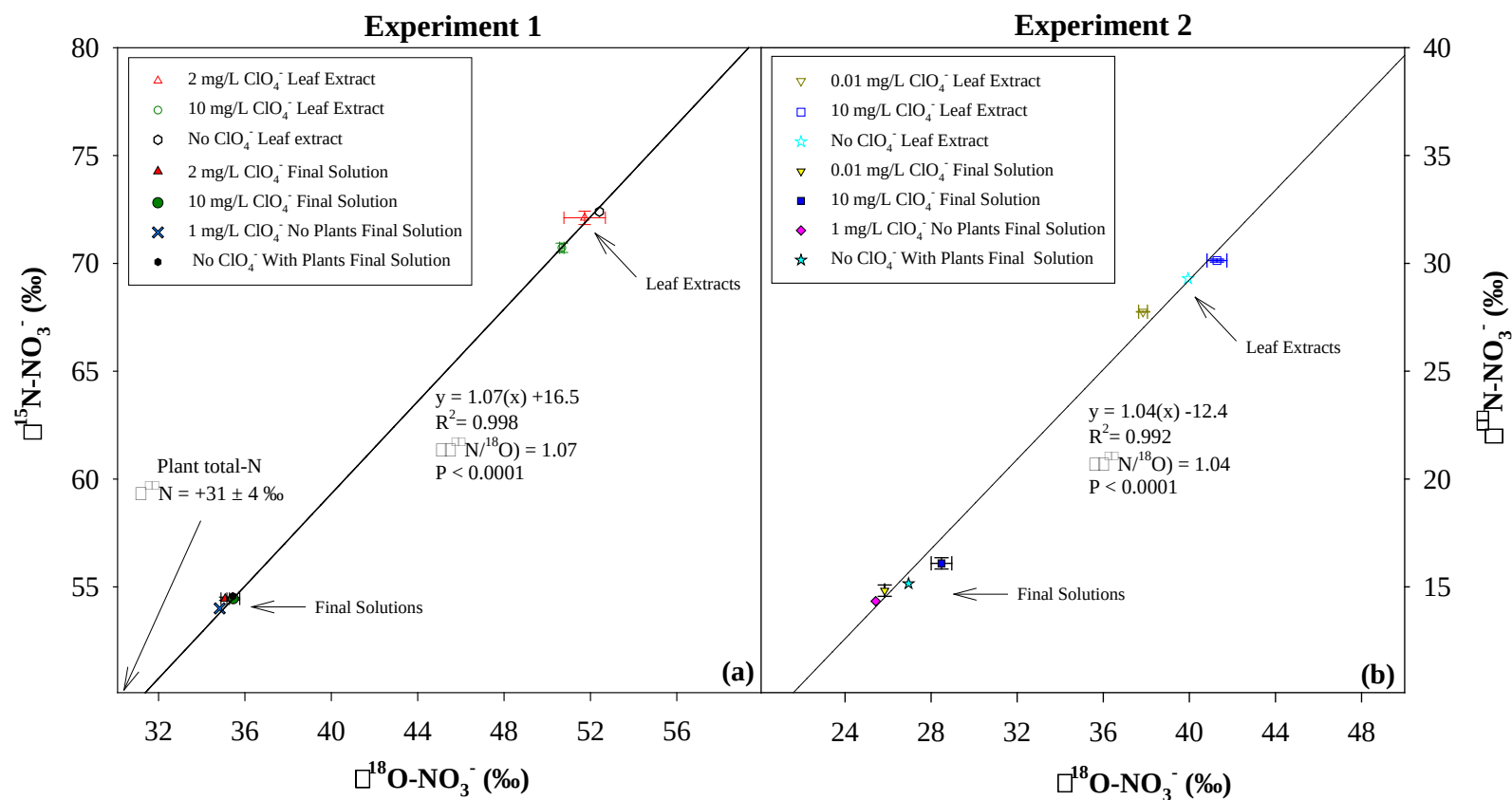


**Figure 2.8.7. Stable isotopic composition of  $\text{NO}_3^-$  in final growth solutions (solid symbols) and leaf extracts (open symbols) of pooled replicate treatments in experiments 1 and 2. Plant total-N  $\delta^{15}\text{N}$  was only measured in experiment 1.**

### 2.8.3.2 Snap Bean Field Study Results

$\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in groundwater used for irrigation and the isotopic composition of indigenous  $\text{ClO}_4^-$  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 ( $< \pm 2\%$ ), to the isotopic composition of  $\text{ClO}_4^-$  in the groundwater. The isotopic composition of  $\text{ClO}_4^-$  in the groundwater was very similar to the composition of Atacama  $\text{ClO}_4^-$ , consistent with the elevated  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  from each source (groundwater and atmospheric deposition) between years based on variations in rainfall and  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in applied groundwater also varied between 2009 and 2010 (0.6 and 2.2  $\mu\text{g/L}$ , respectively), presumably due to exhaustion of the ion exchange column installed to remove  $\text{ClO}_4^-$  from applied groundwater (Table 2.8.3). The increase of applied  $\text{ClO}_4^-$  from groundwater is reflected in the  $\sim 5\text{X}$  higher foliar  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  mass from groundwater in 2010, (48 and 480  $\mu\text{g/m}^2\text{-yr}$  (growing season only), in 2009 and 2010, respectively) (Table 2.8.3). The intermediate isotopic composition of foliar  $\text{ClO}_4^-$  in 2009 is consistent with a larger contribution from indigenous  $\text{ClO}_4^-$  due to the order of magnitude decrease in groundwater applied  $\text{ClO}_4^-$  (i.e., containing Atacama  $\text{ClO}_4^-$ ). Annual average  $\text{ClO}_4^-$  deposition rate in the US is  $\sim 6.5 \mu\text{g/m}^2\text{-yr}$  with an unknown contribution from dry deposition <sup>4</sup>

The  $\text{ClO}_4^-$  composition in snap beans from Long Island appears to reflect multiple sources, possibly dominated by indigenous  $\text{ClO}_4^-$  and Atacama  $\text{ClO}_4^-$  with a smaller contribution of manmade  $\text{ClO}_4^-$  (Figure 2.8.8). It is not possible to determine if the foliar  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  and given the very low concentrations of  $\text{ClO}_4^-$  in the groundwater ( $\sim 0.4 \mu\text{g/L}$ ), the isotopic data are not unreasonable (Böhlke et al., 2009). Regardless, we show that for field-grown plants even with background levels of foliar  $\text{ClO}_4^-$ , it is possible to evaluate foliar isotopic composition.

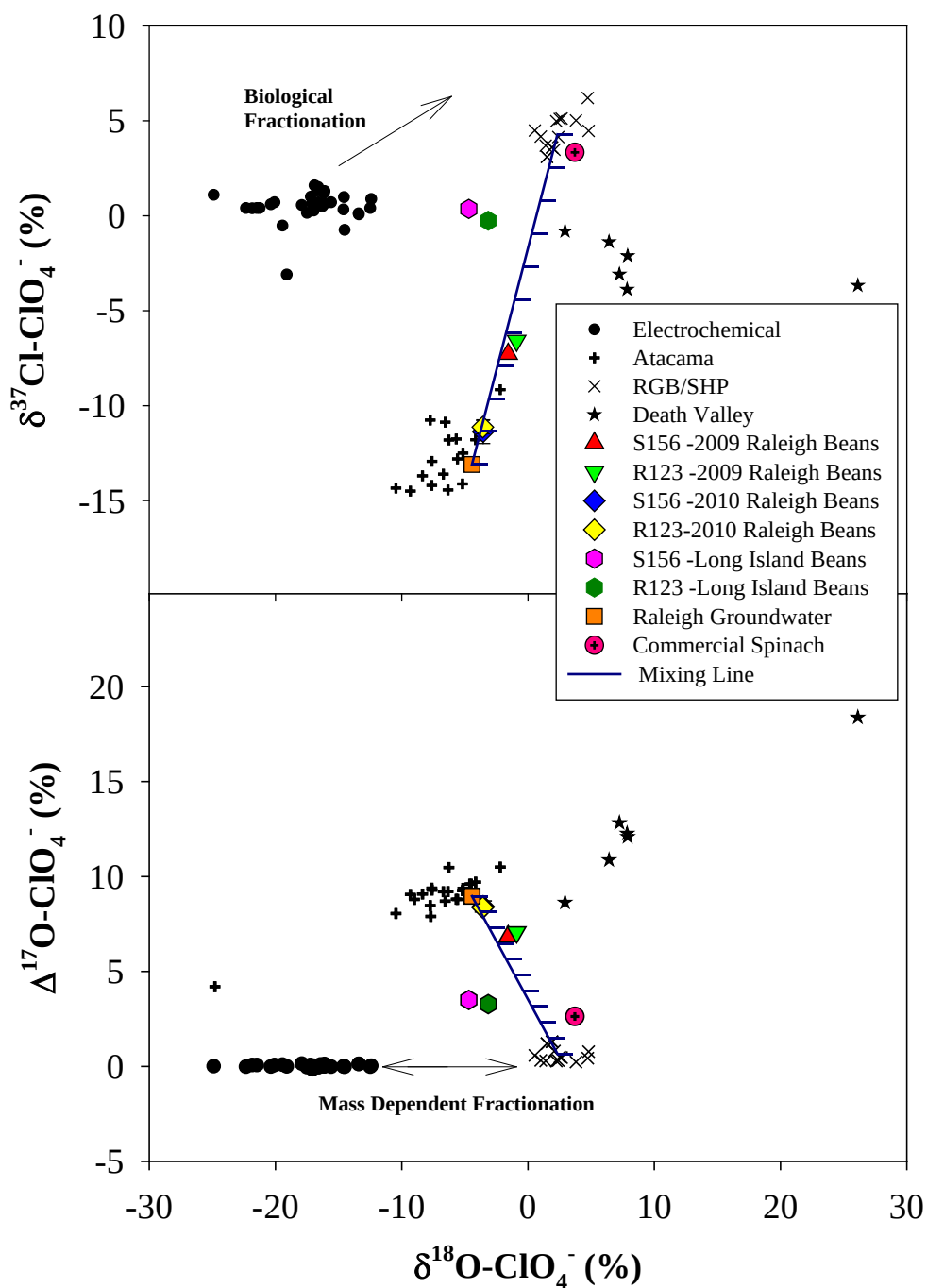


**Figure 2.8.8.** Summary of isotope data for  $\text{ClO}_4^-$  from Raleigh, NC and Long Island, NY field studies and commercial spinach displayed with previously published  $\text{ClO}_4^-$  isotope data from synthetic and natural sources from the Atacama Desert in Chile, Death Valley (DV), California, 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 ( $\pm$  standard deviation). The mixing line (dark blue) was calculated assuming a two-member source (Raleigh groundwater and RGB/SHP) isotopic mass

**Table 2.8.3 Irrigation, rainfall, and foliage  $\text{ClO}_4^-$  data for the Raleigh, NC and Long Island, NY snap bean field studies.** All values reported are mean values.

| Year | Raleigh, NC                              |                     |                                         |                                           |                     |                                         |                                           | Long Island, NY                          |                     |                                         |                                           |                     |                                         |                                           |
|------|------------------------------------------|---------------------|-----------------------------------------|-------------------------------------------|---------------------|-----------------------------------------|-------------------------------------------|------------------------------------------|---------------------|-----------------------------------------|-------------------------------------------|---------------------|-----------------------------------------|-------------------------------------------|
|      | Foliar                                   | Irrigation          |                                         |                                           | Rainfall            |                                         |                                           | Foliar                                   | Irrigation          |                                         |                                           | Rainfall            |                                         |                                           |
|      | $\text{ClO}_4^-$<br>( $\mu\text{g/kg}$ ) | Total<br>Depth (cm) | $\text{ClO}_4^-$<br>( $\mu\text{g/L}$ ) | $\text{ClO}_4^-$<br>( $\mu\text{g/m}^2$ ) | Total<br>Depth (cm) | $\text{ClO}_4^-$<br>( $\mu\text{g/L}$ ) | $\text{ClO}_4^-$<br>( $\mu\text{g/m}^2$ ) | $\text{ClO}_4^-$<br>( $\mu\text{g/kg}$ ) | Total<br>Depth (cm) | $\text{ClO}_4^-$<br>( $\mu\text{g/L}$ ) | $\text{ClO}_4^-$<br>( $\mu\text{g/m}^2$ ) | Total<br>Depth (cm) | $\text{ClO}_4^-$<br>( $\mu\text{g/L}$ ) | $\text{ClO}_4^-$<br>( $\mu\text{g/m}^2$ ) |
| 2009 | 1000                                     | 8.41                | 0.57                                    | 47.94                                     | 24.89               | N/A                                     | N/A                                       |                                          |                     |                                         |                                           |                     |                                         |                                           |
| 2010 | 3000                                     | 21.53               | 2.23                                    | 480.12                                    | 14.25               | N/A                                     | N/A                                       |                                          |                     |                                         |                                           |                     |                                         |                                           |

### 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  $\text{ClO}_4^-$  in food products. The spinach  $\text{ClO}_4^-$  isotopic composition:  $\delta^{37}\text{Cl} = + 3.3 \text{ ‰}$ ,  $\delta^{18}\text{O} = + 3.7 \text{ ‰}$ ,  $\Delta^{17}\text{O} = + 2.6 \text{ ‰}$  was similar to isotopic compositions reported for indigenous natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  stable isotopic composition in soil and groundwater. Uptake of  $\text{ClO}_4^-$  by ion transporters, plant-catalyzed reduction, or oxygen exchange could all potentially cause isotopic fractionation. Snap bean plants were found to selectively uptake  $\text{ClO}_4^-$  relative to  $\text{Cl}^-$ , but not relative to  $\text{NO}_3^-$ , supporting a common ion transport mechanism for  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ . The similarity in uptake rates ( $-0.87$  and  $-0.97 \text{ ‰ mass/L}$ ) for  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ , the strong correlation of anion uptake with volume of transpired solution, and the lower than predicted  $\text{ClO}_4^-$  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  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  but also dependent on transpiration rate (Seyfferth et al., 2008; Marschner, 1995).

No substantial differences were observed in the  $\delta^{37}\text{Cl}$  and  $\delta^{18}\text{O}$  values of  $\text{ClO}_4^-$  between the growth solutions and leaf extracts during the hydroponic experiments. There were small, but inconsistent changes in stable isotopic composition of  $\text{NO}_3^-$  in growth solutions. In experiment 1 a slight increase ( $\sim 1 \text{ ‰}$ ) in  $\delta^{15}\text{N}-\text{NO}_3^-$  but lack of measurable change in  $\delta^{18}\text{O}$  could be consistent with fractionation. The effects of  $\text{NO}_3^-$  reduction or transport processes in the plant on  $\delta^{18}\text{O}$  may not be as apparent as for  $\delta^{15}\text{N}$  due to the difference in analytical uncertainty between the two ( $\sim \pm 1 \text{ ‰}$  versus  $\sim \pm 0.5 \text{ ‰}$ , respectively). In experiment 2, small increases in  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$

in two of the growth solutions, consistent with  $\epsilon^{15}\text{N}$  values around -1.5 ‰ and  $\epsilon^{15}\text{N}/\epsilon^{18}\text{O}$  of around 0.5 – 0.6 might have resulted from isotopic fractionation during uptake into the plants, or they might indicate microbial reduction of  $\text{NO}_3^-$  in solution. If bacterial  $\text{NO}_3^-$  reduction occurred, it does not appear to have been accompanied by substantial  $\text{ClO}_4^-$  reduction based on  $\text{ClO}_4^-$  isotopic compositions.

Although there was a substantial mass of  $\text{ClO}_4^-$  (27-42%) not accounted for in experiment 1, the lack of change in solution  $\text{ClO}_4^-$  stable isotopic composition compared to source material indicates  $\text{ClO}_4^-$  was not a major sink for  $\text{ClO}_4^-$  in growth solutions. If fractionation of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  could also cause mass-dependent fractionation of  $\text{ClO}_4^-$ . While in experiment 1 approximately 27-42% of the  $\text{ClO}_4^-$  was unaccounted for, in experiment 2 and in other published studies,  $\text{ClO}_4^-$  generally appears to have been conserved in plant-water systems. In both experiments, essentially identical  $\text{ClO}_4^-$  stable isotopic composition in leaf tissue, growth solution, and source  $\text{ClO}_4^-$  makes it unlikely that substantial transformation of  $\text{ClO}_4^-$  occurred. Only one study, using hybrid poplar trees (*P. deltoids x nigra*) and radio labeled  $\text{ClO}_4^-$  ( $^{36}\text{ClO}_4^-$ ), gave evidence of  $\text{ClO}_4^-$  metabolism within plant tissue (Van Aken et al., 2002). In that study approximately 10 – 34 % of the radioactivity ( $^{36}\text{Cl}$ ) introduced as  $\text{ClO}_4^-$  was recovered as  $\text{ClO}_3^-$ ,  $\text{ClO}_2^-$ , and  $\text{Cl}^-$ . Our results, and other published studies (Seyfferth et al., 2008; Nzengung et al., 2003; Tan et al., 2004; Suslara et al., 2000; Ha et al., 2011) suggest that plant-mediated transformation may not be common. Our results further indicate no measurable plant-mediated exchange of O occurred between  $\text{ClO}_4^-$  and other compounds such as  $\text{H}_2\text{O}$  (i.e.,  $\leq 1 - 2$  % exchange in a growing season).

In contrast to  $\text{ClO}_4^-$ ,  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  in plant tissue was fractionated substantially (~10-20‰), consistent with reported fractionation effects of  $\text{NO}_3^-$  assimilation in marine culture studies (~15-30‰) (Needoba et al., 2004; Granger et al., 2010; Ledgard et al., 1985; Schmidt and Medina, 1991). The  $\epsilon^{15}\text{N}/\epsilon^{18}\text{O}$  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  $\epsilon^{15}\text{N}/\epsilon^{18}\text{O}$  ratio of approximately 1, regardless of the magnitudes of  $\epsilon^{15}\text{N}$  and  $\epsilon^{18}\text{O}$  values expressed (Karsh et al., 2012, 2014; Granger et al., 2004).

We also demonstrate that field-grown plants exposed to environmentally relevant  $\text{ClO}_4^-$  concentrations do not appear to affect foliar  $\text{ClO}_4^-$  isotopic composition.  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$ , 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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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). The lower concentrations of  $\text{ClO}_4^-$  in spinach, compared to those in the hydroponic bean plants, are consistent with exposure to low level background  $\text{ClO}_4^-$  commonly found in groundwater (MRGB: 0.12 to 0.12 to 1.8  $\mu\text{g/L}$  ; SHP:  $\sim 0.1$  to 200  $\mu\text{g/L}$ ) or soil (SHP: 3.3  $\mu\text{g/kg}$ ) (Jackson et al., 2010). Because the spinach  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  isotopic composition represents the source  $\text{ClO}_4^-$  isotopic composition. However, it is also possible that  $\text{ClO}_4^-$  in spinach tissue was altered isotopically (e.g. reduction or oxygen exchange). If so, while the O isotopic composition of spinach  $\text{ClO}_4^-$  might be relatively independent of the source values, the Cl isotopic composition of spinach  $\text{ClO}_4^-$  might still reflect the source value, which would be consistent with an indigenous  $\text{ClO}_4^-$  source and not electrochemical or Atacama  $\text{ClO}_4^-$ .

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  $\text{ClO}_4^-$  in commercial produce and in other plant-based food products. This finding is important because most of the exposure to  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in plant tissue may provide a direct method to distinguish the dominant sources of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  uptake and accumulation can occur without significant net loss or isotope effects and that plant  $\text{NO}_3^-$  reduction systems efficiently exclude  $\text{ClO}_4^-$ , in contrast to some other  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in the food chain, as well as to determine if other types of plants in other settings can alter the isotopic composition of  $\text{ClO}_4^-$ .

## 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.

1. Jackson, W.A., J.K. Bohlke, B. J. Andraski, L. Fahlquist, L. Bexfield, F.D. Eckardt, J. B. Gates, A.F. Davila, C.P. McKay, B. Rao, R. Sevanthi, S. Rajagopalan, N. Estrada<sup>1</sup>, N. C. Sturchio, P.B. Hatzinger, T. A. Anderson, G. Orris, J. Betancourt, D. Stonestrom, C. Latorre, Y. Li, and G. Harvey. 2015. Global survey of perchlorate and nitrate co-occurrence in arid and semi-arid regions. *Geochim. Cosmochim. Acta* 164:502-522.
2. Poghosyan, A., N.C. Sturchio, C.G. Morrison, Y. Guan, J.M. Eiler, W.A. Jackson, P.B. Hatzinger. 2014. Perchlorate in the Great Lakes: Isotopic composition and origin. *Environ. Sci. Technol.* 48:11146-11153.
3. Jackson, W.A., J.K. Böhlke, B. Gu, P.B. Hatzinger and N.C. Sturchio. 2010. Isotopic composition and origin of indigenous natural perchlorate and co-occurring nitrate in the southwestern United States. *Environ. Sci. Technol.* 44:4869-4876.
4. Grantz, D.A., K.O. Burkey, W.A. Jackson, H-B Vu, M.T. McGrath, and G. Harvey. 2014. Perchlorate content of plant foliage reflects a wide range of species-dependent accumulation but not ozone-induced biosynthesis. *Environ. Pollut.* 184:690-696.
5. Grantz, D.A., A. Jackson, H-B Vu, K.O. Burkey, M.T. McGrath, and G. Harvey. 2014. High ozone increases soil perchlorate but does not affect foliar perchlorate content. *J. Environ. Qual.* 43:1460-1466.
6. Jackson, W.A., A. F. Davila, N. Estrada, W. Berry Lyons, J. D. Coates, J.C. Prisco. 2012. Perchlorate and chlorate biogeochemistry in ice-covered lakes of the McMurdo Dry Valleys, Antarctica. *Geochim. Cosmochim. Acta* 98: 19-30.
7. Estrada, N.L. 2015. Effects of Plant Uptake and UV and O<sub>3</sub> Production Mechanisms on Perchlorate Isotopic Composition and Possible Implications to Natural Perchlorate. Ph.D. Dissertation, Texas Tech University, 230 pg.

### 3.0 CONCLUSIONS and IMPLICATIONS FOR FUTURE RESEARCH

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

Worldwide soil and groundwater sampling data indicate that  $\text{ClO}_4^-$  is globally distributed in soil and groundwater in arid and semi-arid regions on Earth at concentrations ranging from  $10^{-1}$  to  $10^6$   $\mu\text{g/kg}$ . Generally, the  $\text{ClO}_4^-$  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,  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  co-occur at consistent ratios ( $\text{NO}_3^-/\text{ClO}_4^-$ ) that vary between  $\sim 10^4$  and  $\sim 10^5$ . This is not the case for  $\text{Cl}^-/\text{ClO}_4^-$  ratios, which vary widely among locations. The  $\text{NO}_3^-/\text{ClO}_4^-$  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  $\text{ClO}_4^-$  and  $\text{NO}_3^-$  in arid and semi-arid locations, and associated variations in the isotopic composition of the  $\text{NO}_3^-$ , are consistent with a conceptual model of atmospheric origin, global co-deposition, and variable alteration of the  $\text{NO}_3^-$  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  $\text{ClO}_4^-$  compared to  $\text{Cl}^-$  or  $\text{NO}_3^-$ , accompanied by unique  $\text{ClO}_4^-$  isotopic characteristics, may reflect an unusually efficient, but yet unknown, *in situ* production mechanism, regionally elevated atmospheric  $\text{ClO}_4^-$  production rates, or higher  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  isotopic characteristics and high relative  $\text{ClO}_4^-$  concentrations in the Atacama.

Stable isotope analysis of Cl and O and radioactive isotope analysis of  $^{36}\text{Cl}$  in natural  $\text{ClO}_4^-$  confirmed and extended initial data suggesting that indigenous  $\text{ClO}_4^-$  sources in the southwestern U.S. show some isotopic variation by location and environment but remain isotopically distinct from synthetic and Atacama  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  concentrations ranging from 0.05 to 0.13  $\mu\text{g/L}$  (varying by lake) with concentrations being nearly constant with depth. Interestingly, the overall ranges of stable isotopic compositions of Great Lakes  $\text{ClO}_4^-$  resemble those of indigenous natural  $\text{ClO}_4^-$  measured

in groundwaters of the western USA indicating a predominantly natural atmospheric source of  $\text{ClO}_4^-$  in all of the lakes.

$\text{ClO}_4^-$  and  $\text{ClO}_3^-$  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  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  biotransformation in pristine extreme environments. Given their low concentrations, high solubility, and lack of any *in situ* generation mechanisms,  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  may offer a sensitive means to study ongoing biological activity in the lakes, and the addition of  $\text{ClO}_4^-$  stable isotope evaluation could provide further clues as to the geochemical history of the lake water. Finally,  $\text{ClO}_3^-$  and  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  in the Martian soil. Further research with Antarctic  $\text{ClO}_4^-$  samples, including isotopic analysis is recommended.

Several potential biological mechanisms of  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  samples. Bacterial production of  $\text{ClO}_4^-$  was assessed using (1) various nitrifying cultures and enrichments that oxidize  $\text{NH}_4$  to  $\text{NO}_3^-$ ; (2) natural haloperoxidase enzymes that are known to oxidize  $\text{Cl}^-$  to hypochlorous acid ( $\text{HClO}$ ) and potentially to  $\text{ClO}_4^-$  (possibly via additional photochemical or biological reactions); and (3) organisms capable of oxidizing sulfite or phosphite. A variety of experiments were conducted with  $\text{Cl}^-$  or  $\text{ClO}_x$  precursors in the presence of different enzymes, organisms, and conditions as summarized above. While some  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  production. The other organisms and processes evaluated did not result in  $\text{ClO}_4^-$  formation.

A variety of plant species were also evaluated for their potential to accumulate and even generate  $\text{ClO}_4^-$  via  $\text{O}_3$ -mediated processes. A broad range of crop species was observed to accumulate  $\text{ClO}_4^-$  from growth medium, and these species differed widely in their bioconcentration of the anion. Foliar  $\text{ClO}_4^-$  concentration was greatest in older leaves, which ultimately contribute to the litter layer, suggesting that scavenging of  $\text{ClO}_4^-$  from deeper soil horizons could lead to redistribution on the soil surface. However, there was no evidence that exposure of leaves to ambient  $\text{O}_3$  or at significantly elevated  $\text{O}_3$  induced any increase in tissue contents of  $\text{ClO}_4^-$ . The results indicate that  $\text{O}_3$  does not lead to increased phyto-accumulation nor plant biosynthesis of  $\text{ClO}_4^-$ .

The impact of plant accumulation of  $\text{ClO}_4^-$  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  $\delta^{37}\text{Cl}$ ,  $\delta^{18}\text{O}$ , or  $\Delta^{17}\text{O}$  values of  $\text{ClO}_4^-$  between the growth solutions and leaf extracts. In contrast to  $\text{ClO}_4^-$ ,  $\delta^{15}\text{N}$

of  $\text{NO}_3^-$  in plant tissue was fractionated substantially ( $\sim 10\text{-}20\%$ ). The  $\epsilon^{15}\text{N}/\epsilon^{18}\text{O}$  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  $\text{ClO}_4^-$  similarly to  $\text{NO}_3^-$ .

Similar to hydroponically grown plants, field grown plants exposed to environmentally relevant  $\text{ClO}_4^-$  concentrations also did not appear to affect foliar  $\text{ClO}_4^-$  isotopic composition.  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  stable isotopes. The spinach had an isotopic composition similar to that of indigenous natural  $\text{ClO}_4^-$  from the SHP as well as the Great Lakes. This result could indicate the spinach was exposed to natural  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  (i.e., synthetic, Atacama, indigenous) in commercial produce and in other plant-based food products through stable isotope analysis of plant accumulated  $\text{ClO}_4^-$ . This finding is important because most of the exposure to  $\text{ClO}_4^-$  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  $\text{ClO}_4^-$  isotope signatures in vegetation reflect those in irrigation water and/or local soils and groundwater, thus allowing forensic determination of  $\text{ClO}_4^-$  sources in food crops.

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

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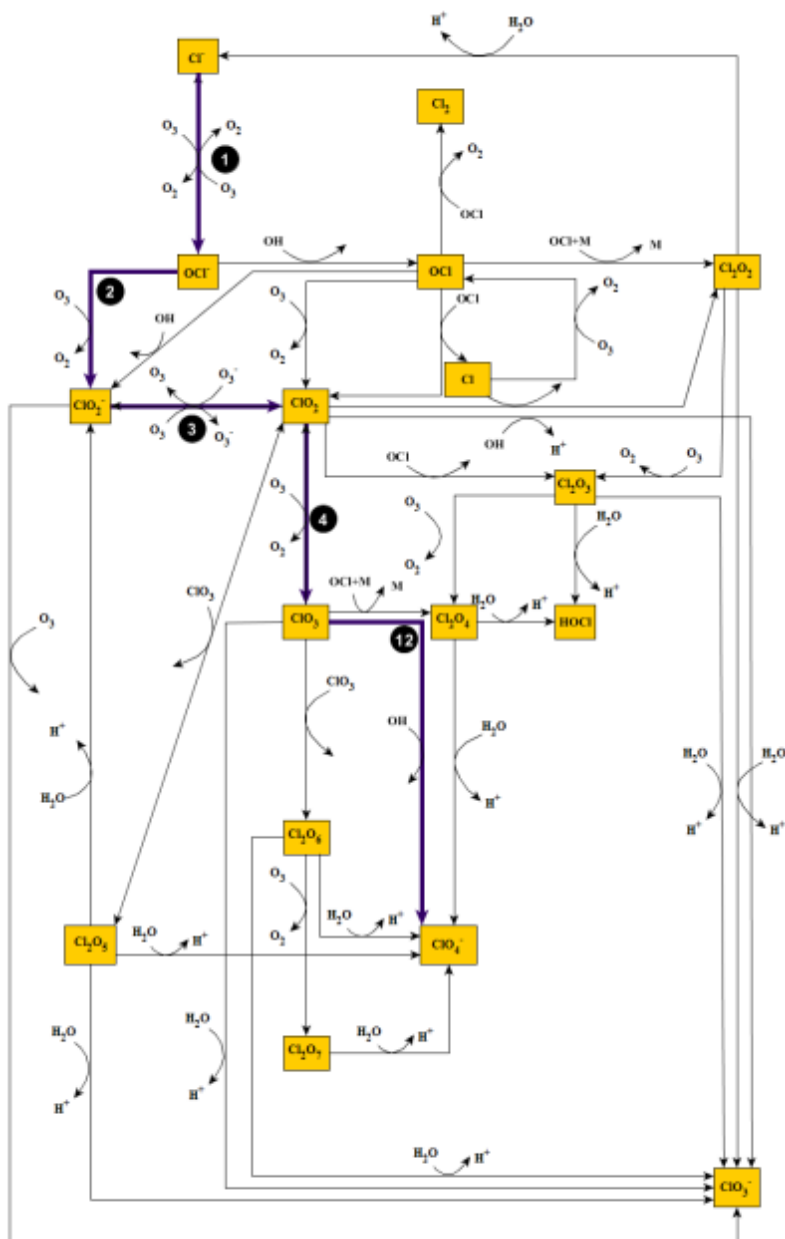
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## **Appendix A: Proposed Mechanisms of Perchlorate Formation**

## A.1 Proposed Pathways for O<sub>3</sub> Oxidation of Aqueous Chloride

### A.1.1 ClO<sub>4</sub><sup>-</sup> formed from ClO<sub>3</sub> oxidation by OH radical (Final Proposed Pathways)



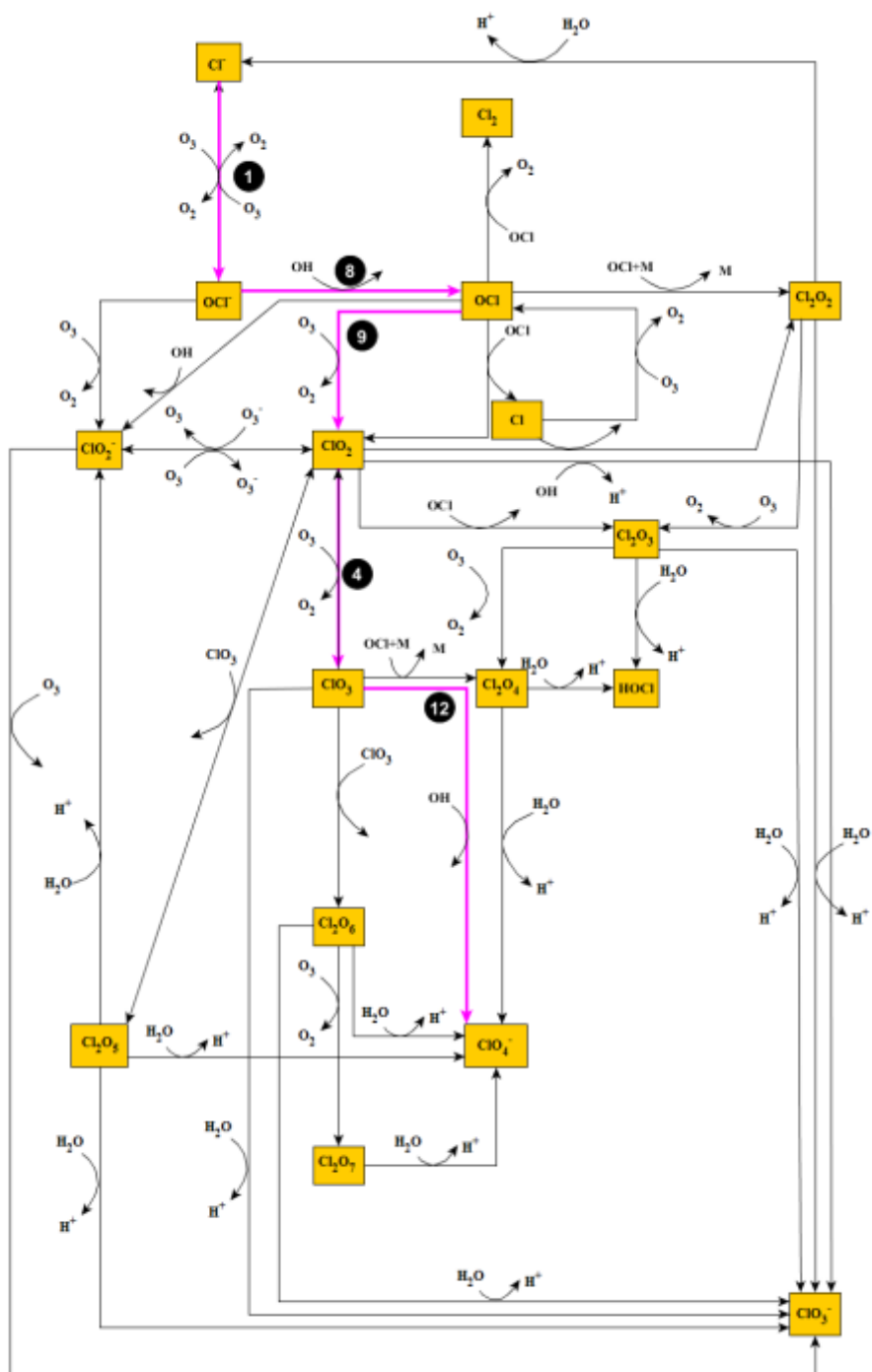


**Figure A.2 Mechanism 2:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq).** Note the thick pink lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule one containing four O's from  $\text{O}_3$ .

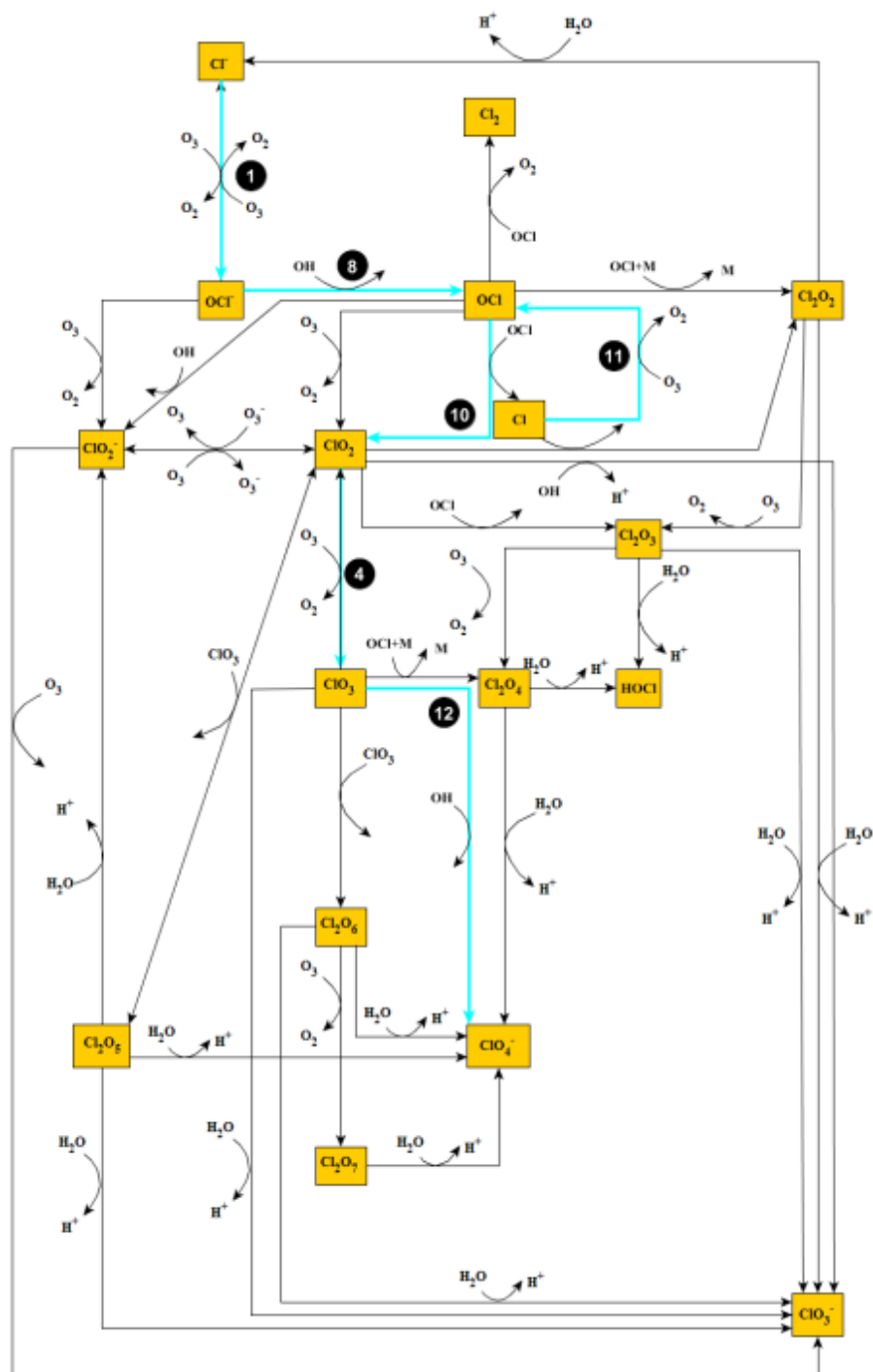


Figure A.3 Mechanism 3.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick aqua blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing all four O's from  $\text{O}_3$ .



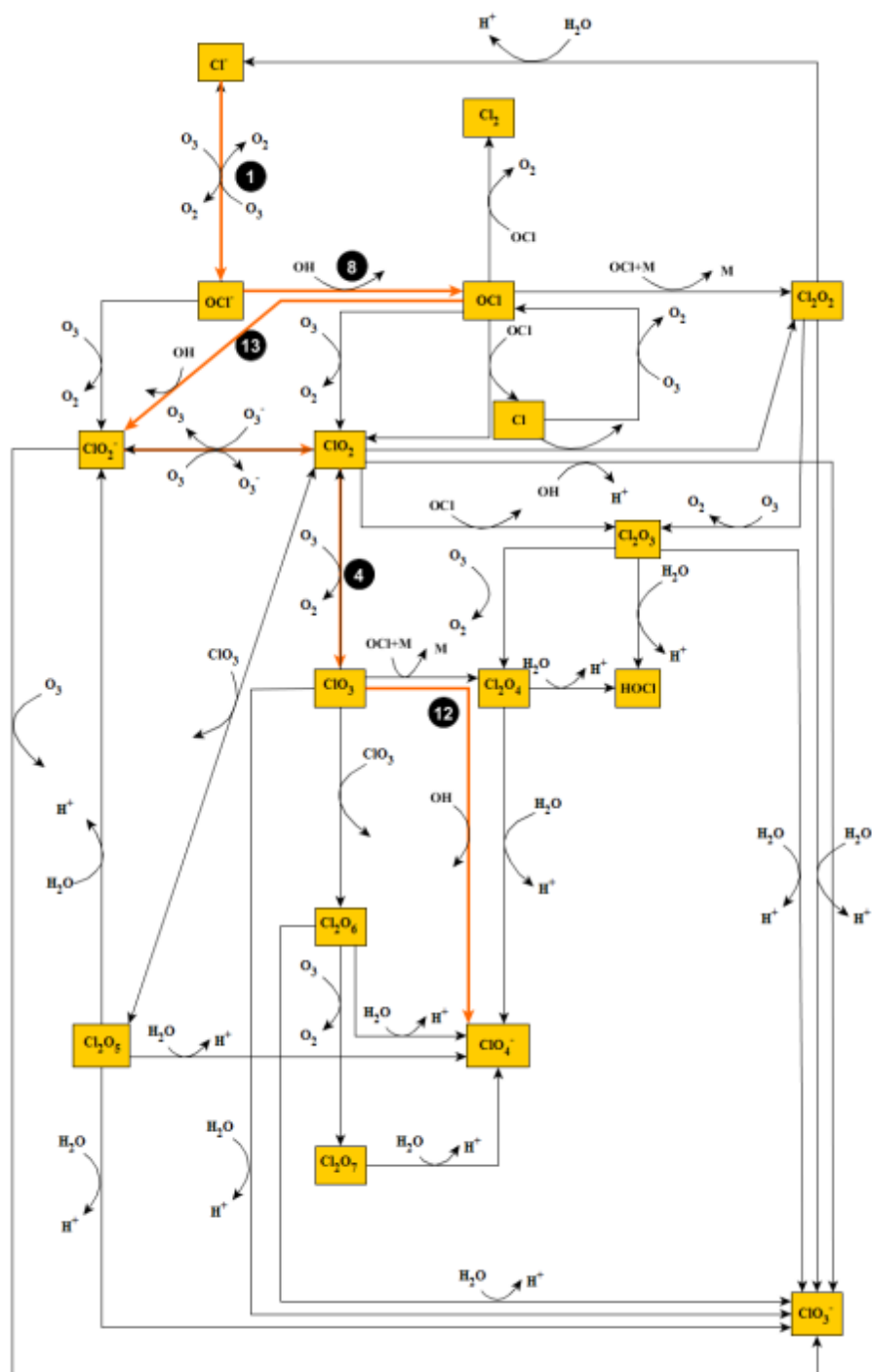


**Figure A.4 Mechanism 4.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick orange lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing all four O's from  $\text{O}_3$ .**

### A.1.2 $\text{ClO}_4^-$ Formed from Hydrolysis of the $\text{Cl}_2\text{O}_7$ Species

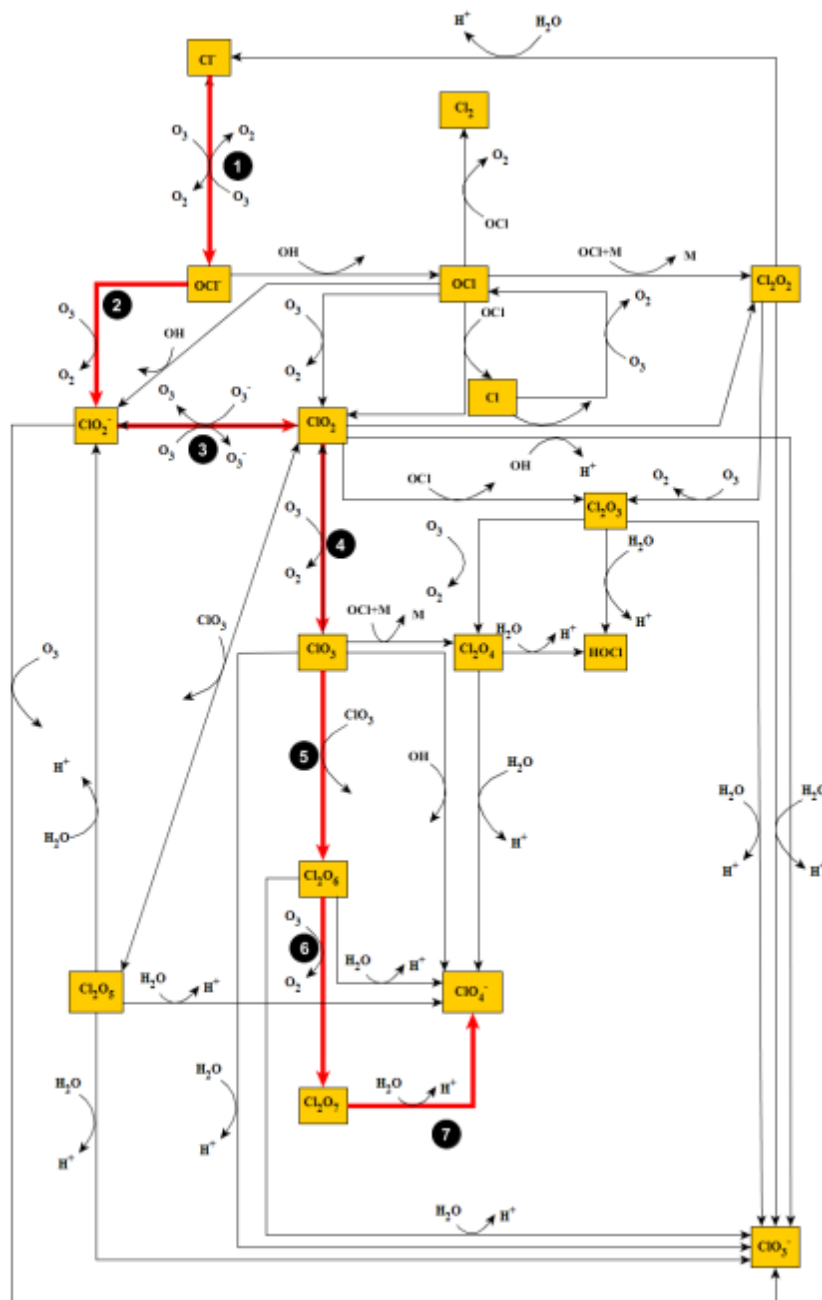


Figure A.5 Mechanism 5:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick red lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$  molecules, one with three O from  $\text{O}_3$  and the other with four O from  $\text{O}_3$ .

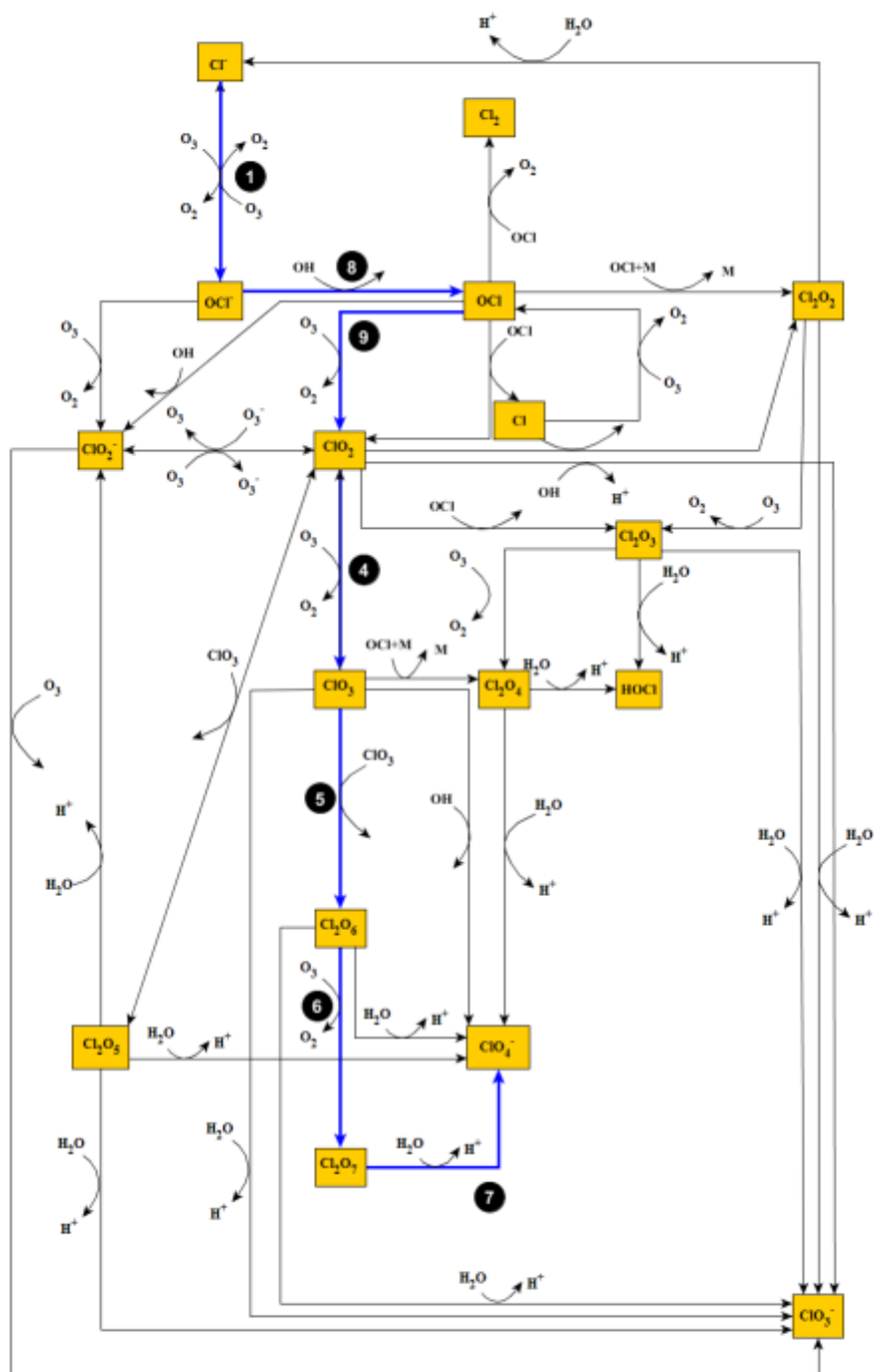


Figure A.6 Mechanism 6:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$  molecules one containing three O's from  $\text{O}_3$  and the other four O's from  $\text{O}_3$ .

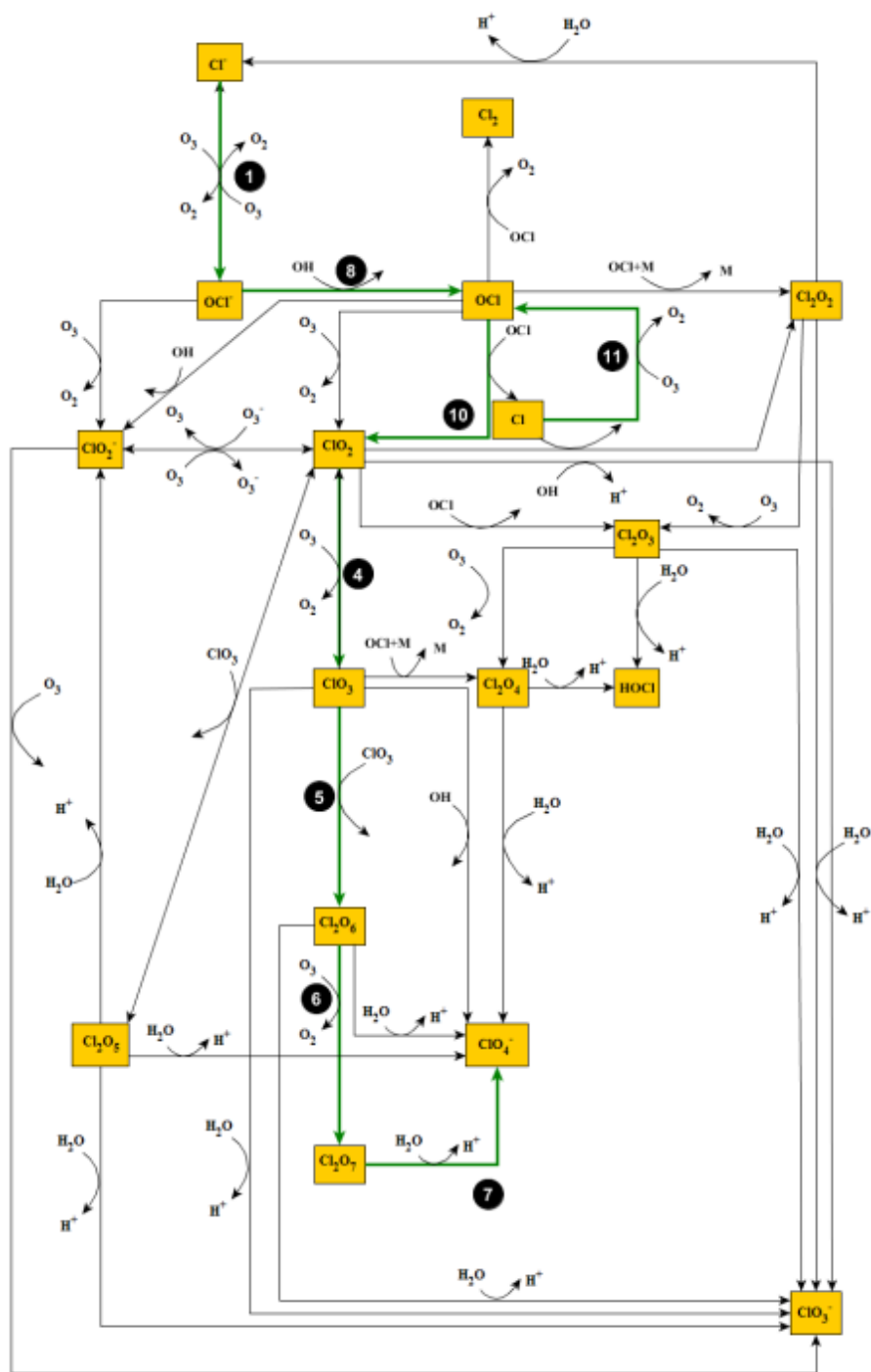


Figure A.7 Mechanism 7.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick apple green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$  molecules, one with three O from  $\text{O}_3$  and the other with four O from  $\text{O}_3$ .

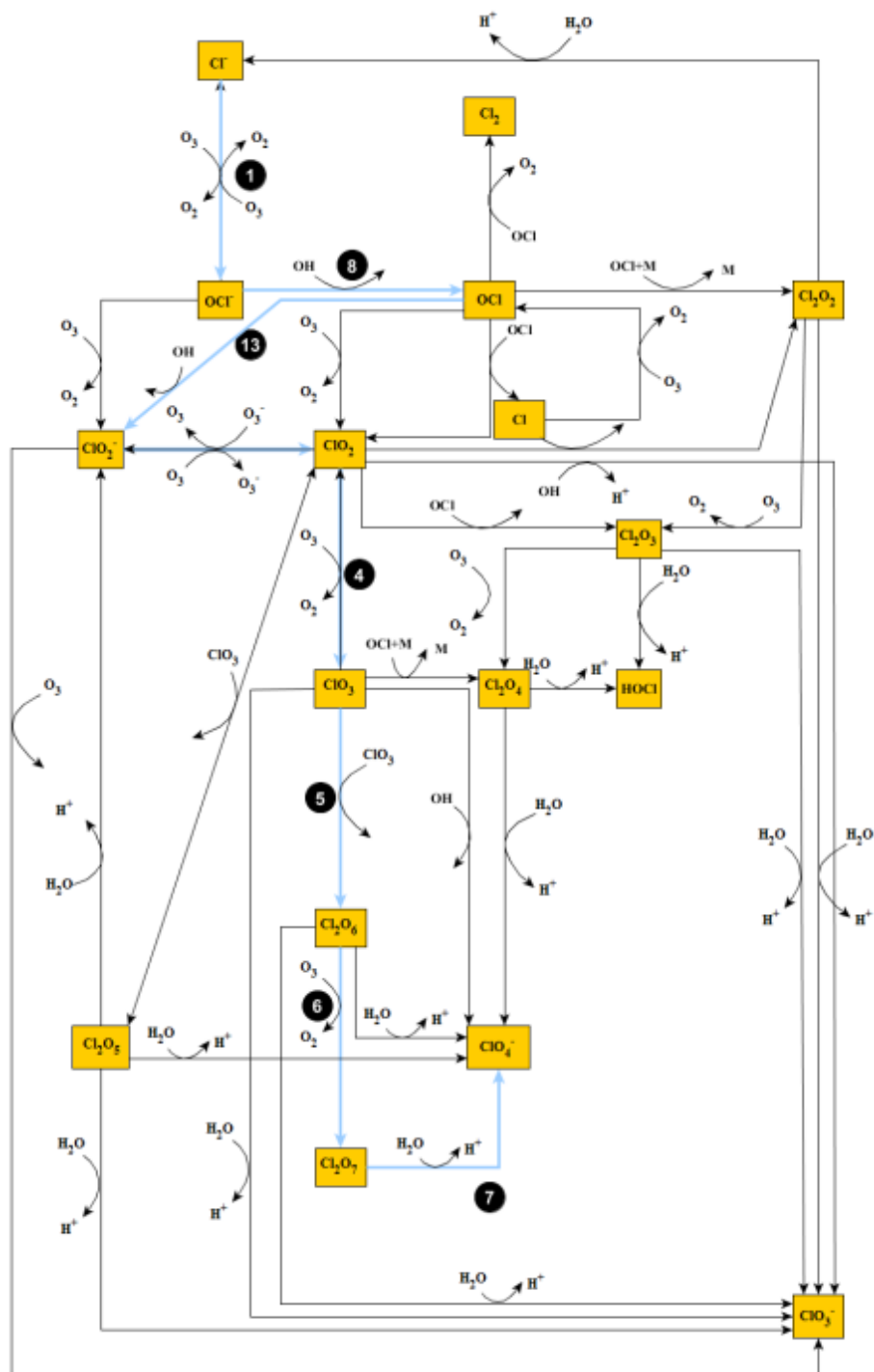


Figure A.8 Mechanism 8.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick light blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$  molecules, one with three O from  $\text{O}_3$  and the other with four O from  $\text{O}_3$ .

### A.1.3 Pathways that Produce $\text{ClO}_4^-$ with Only 3 O atoms from $\text{O}_3$

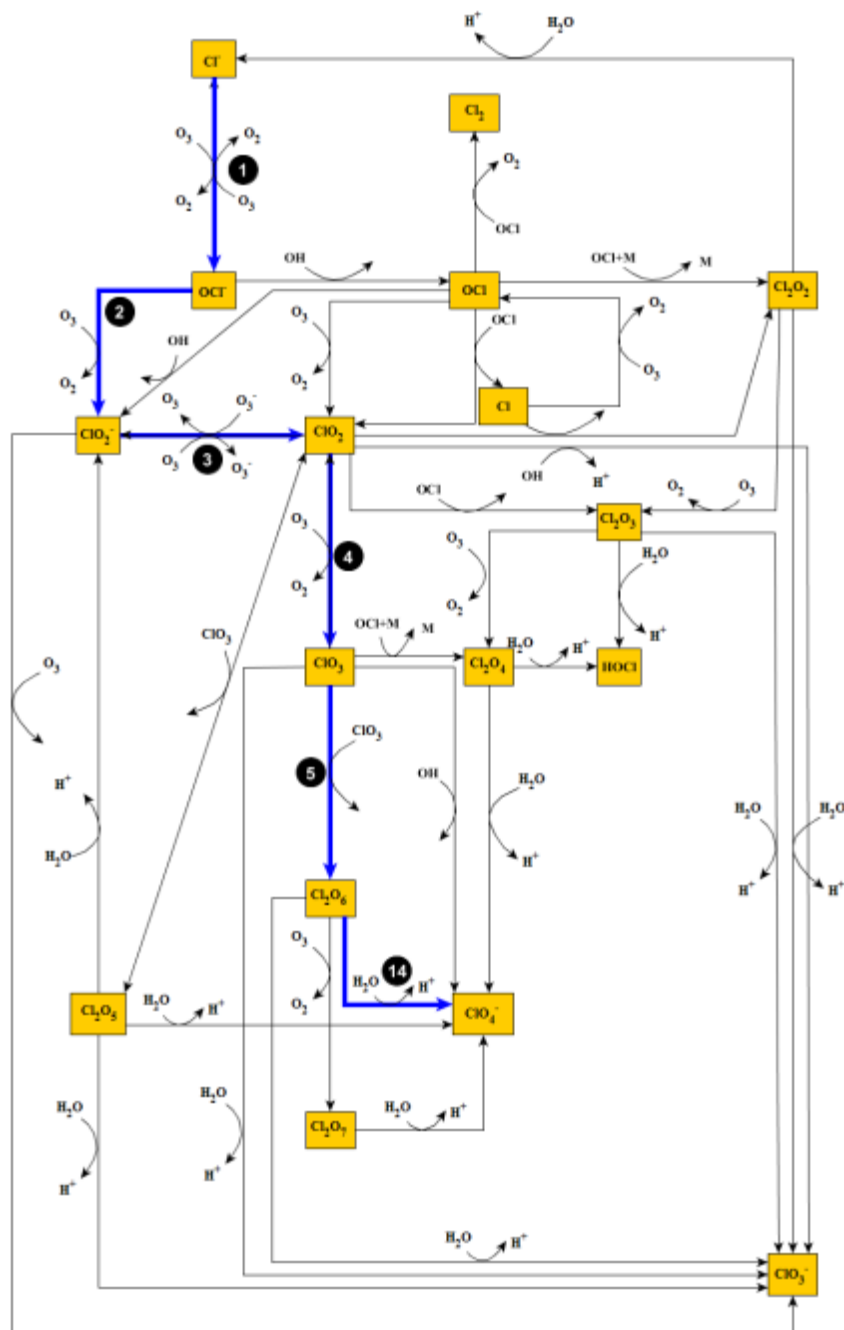


Figure A.9 Mechanism 9:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$  and a  $\text{ClO}_3^-$  molecule containing all three O's from  $\text{O}_3$ .

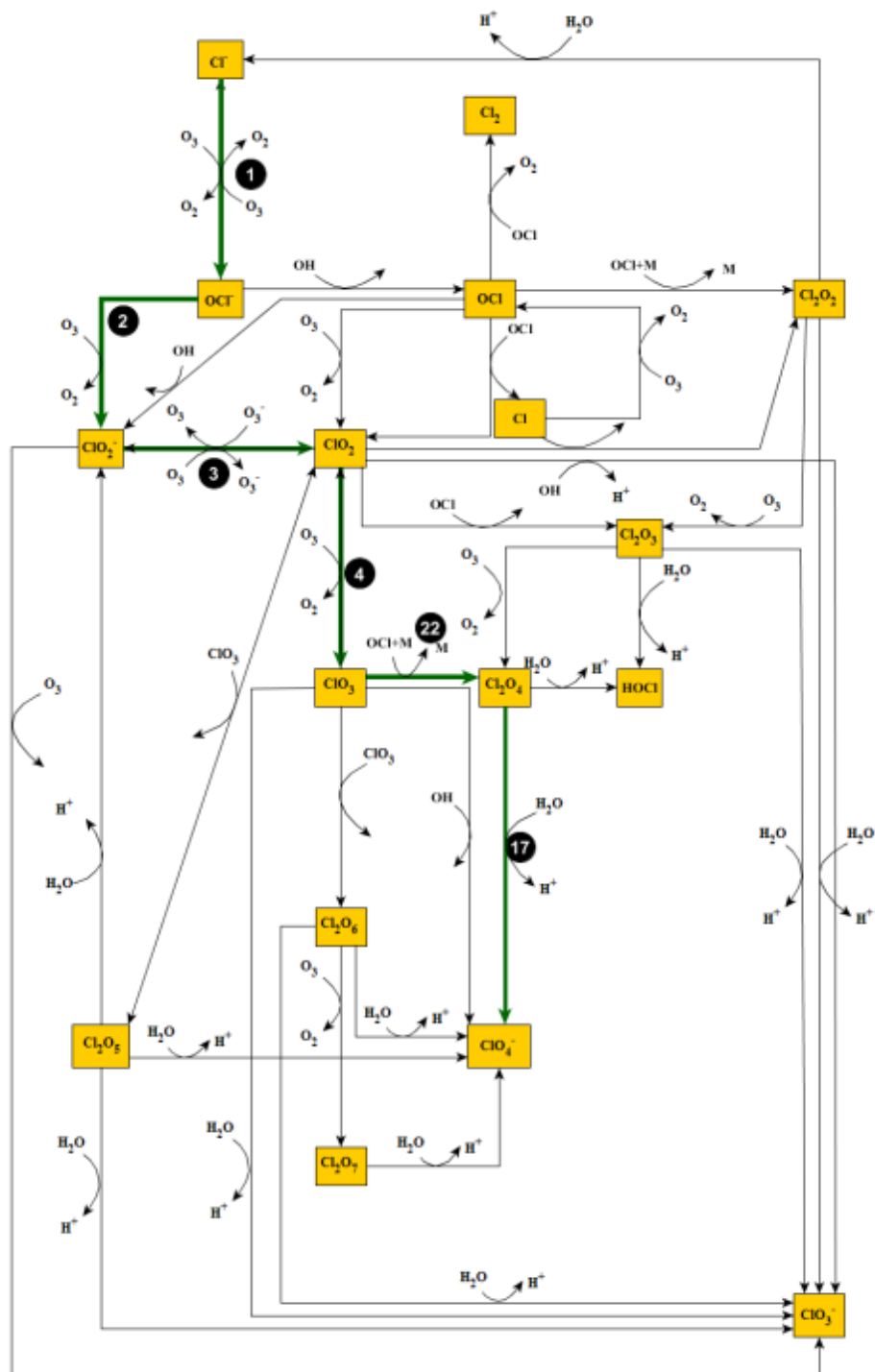


Figure A.10 Mechanism 10:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

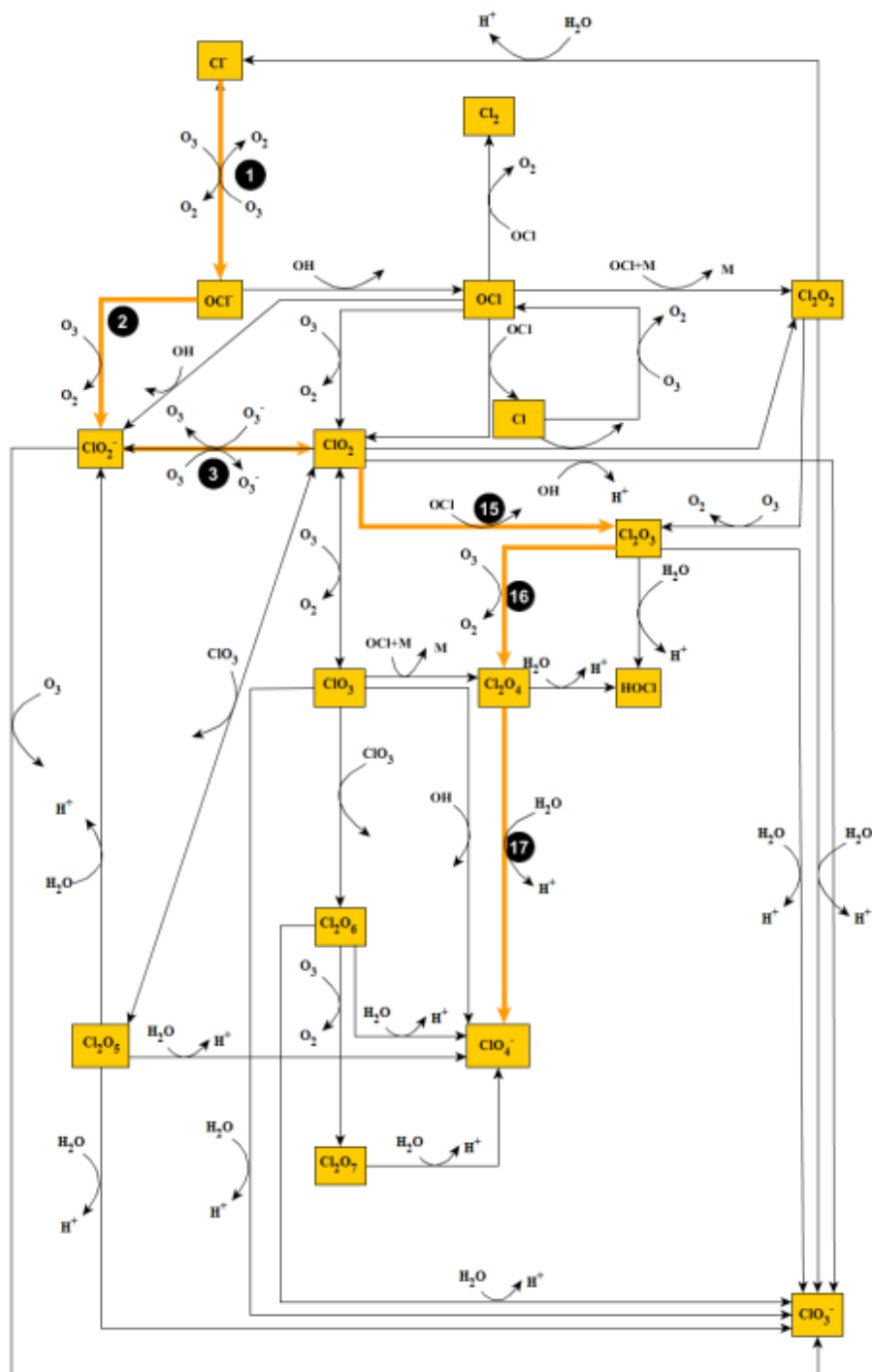


**Figure A.11 Mechanism 11:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick orange lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .**



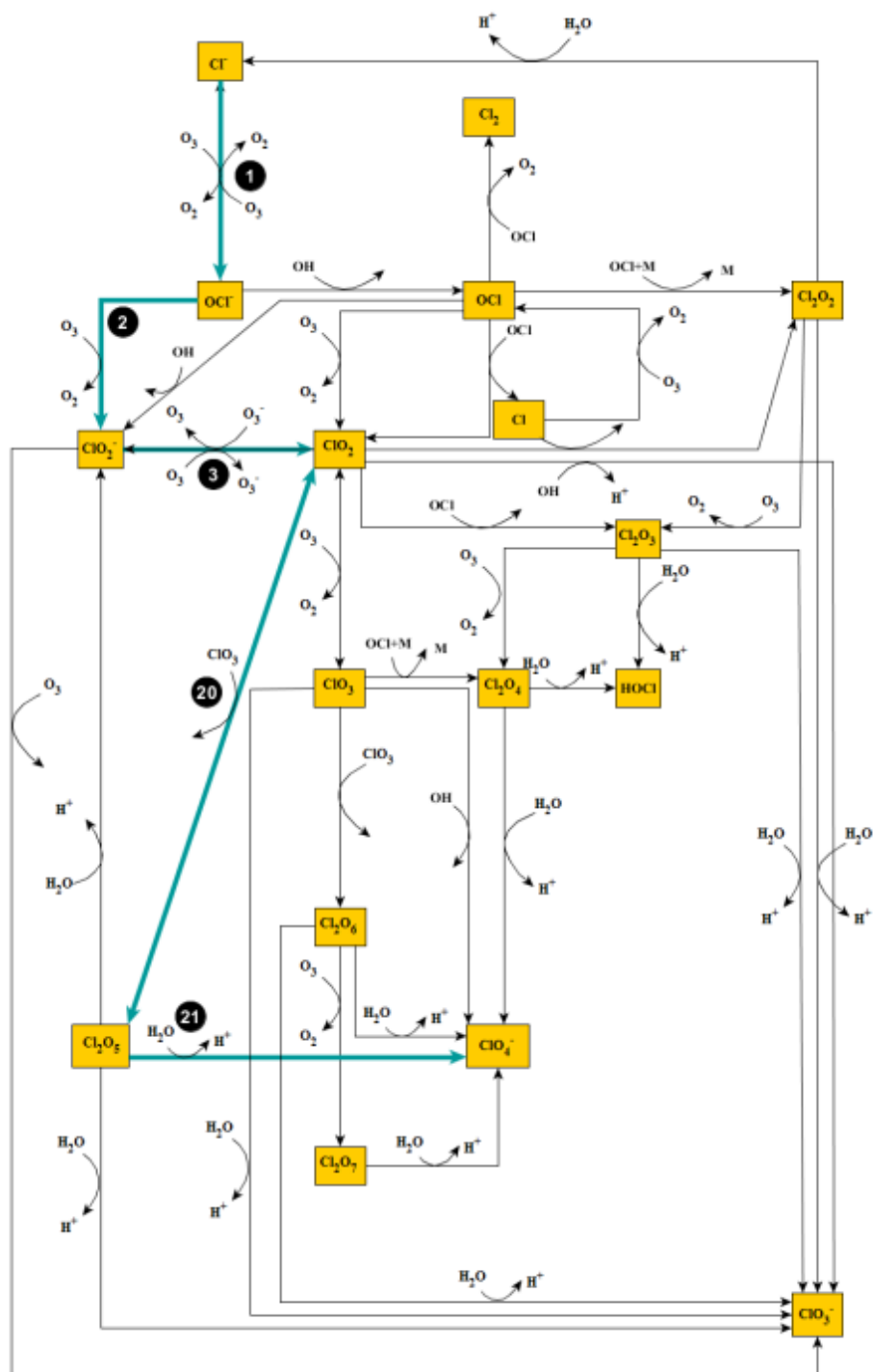


**Figure A.12 Mechanism 12:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq).** Note the thick aquamarine lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

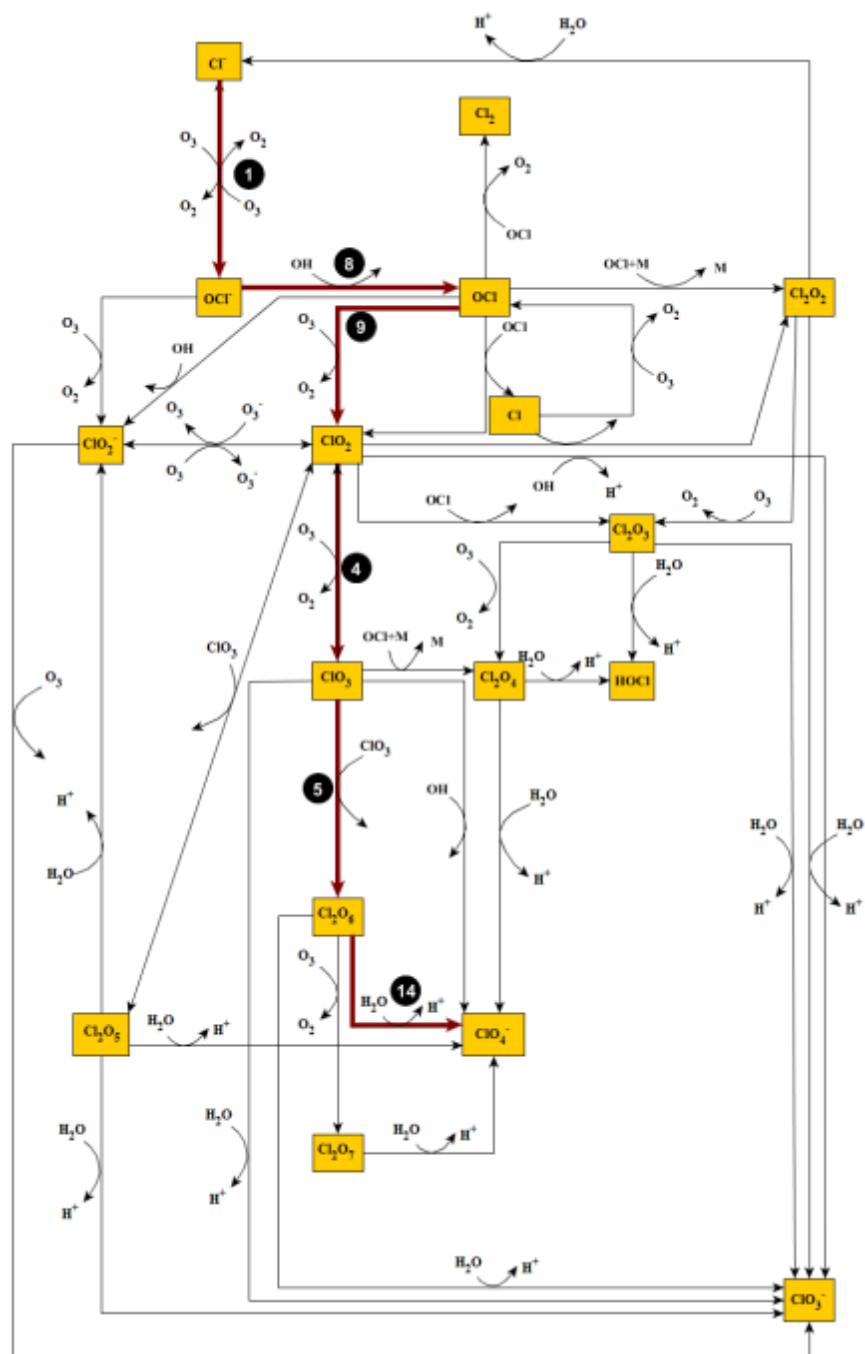


Figure A.13 Mechanism 13:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick maroon lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$  and a  $\text{ClO}_3^-$  molecule containing all three O's from  $\text{O}_3$ .



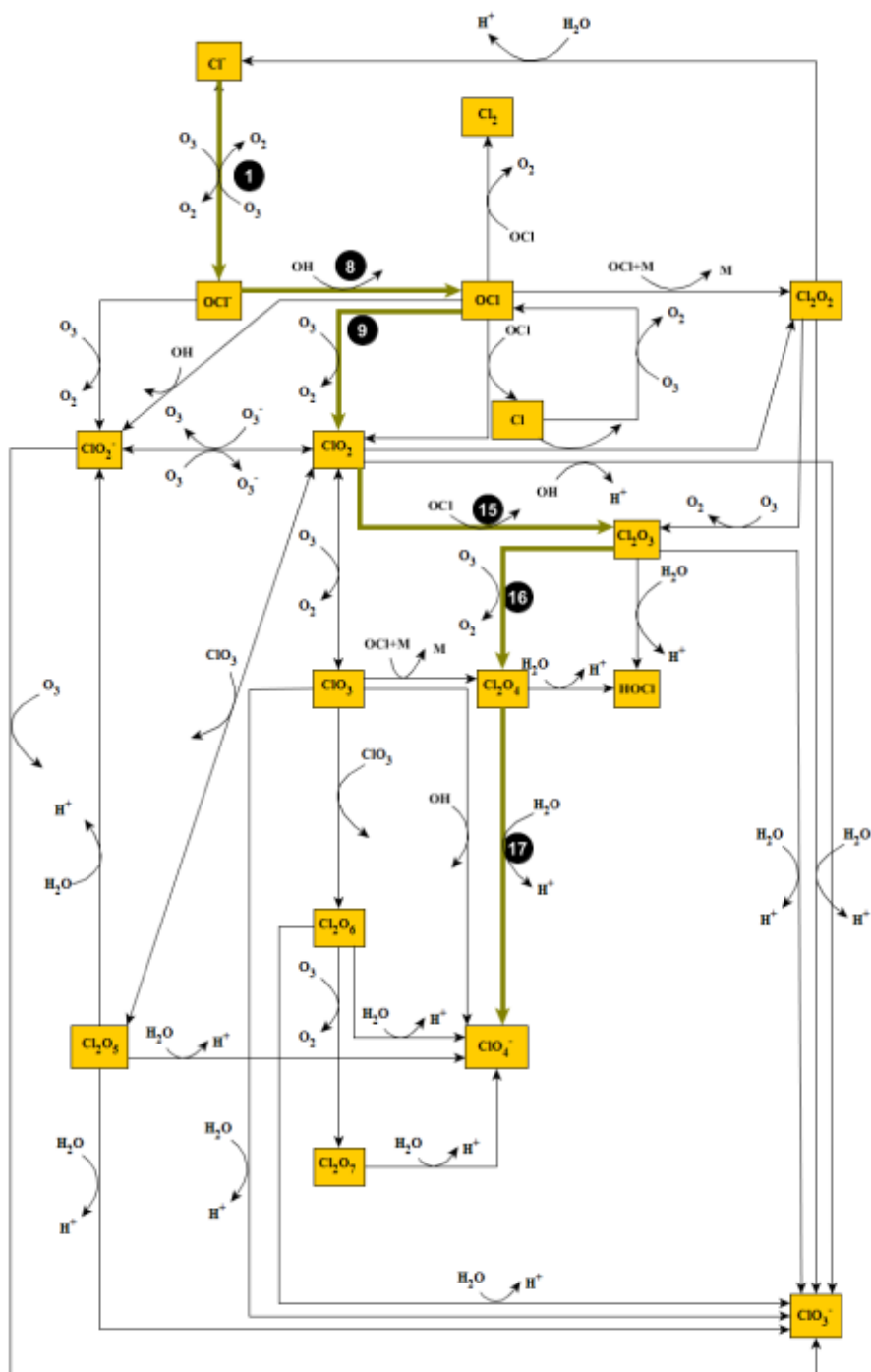


Figure A.15 Mechanism 15:  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick olive green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

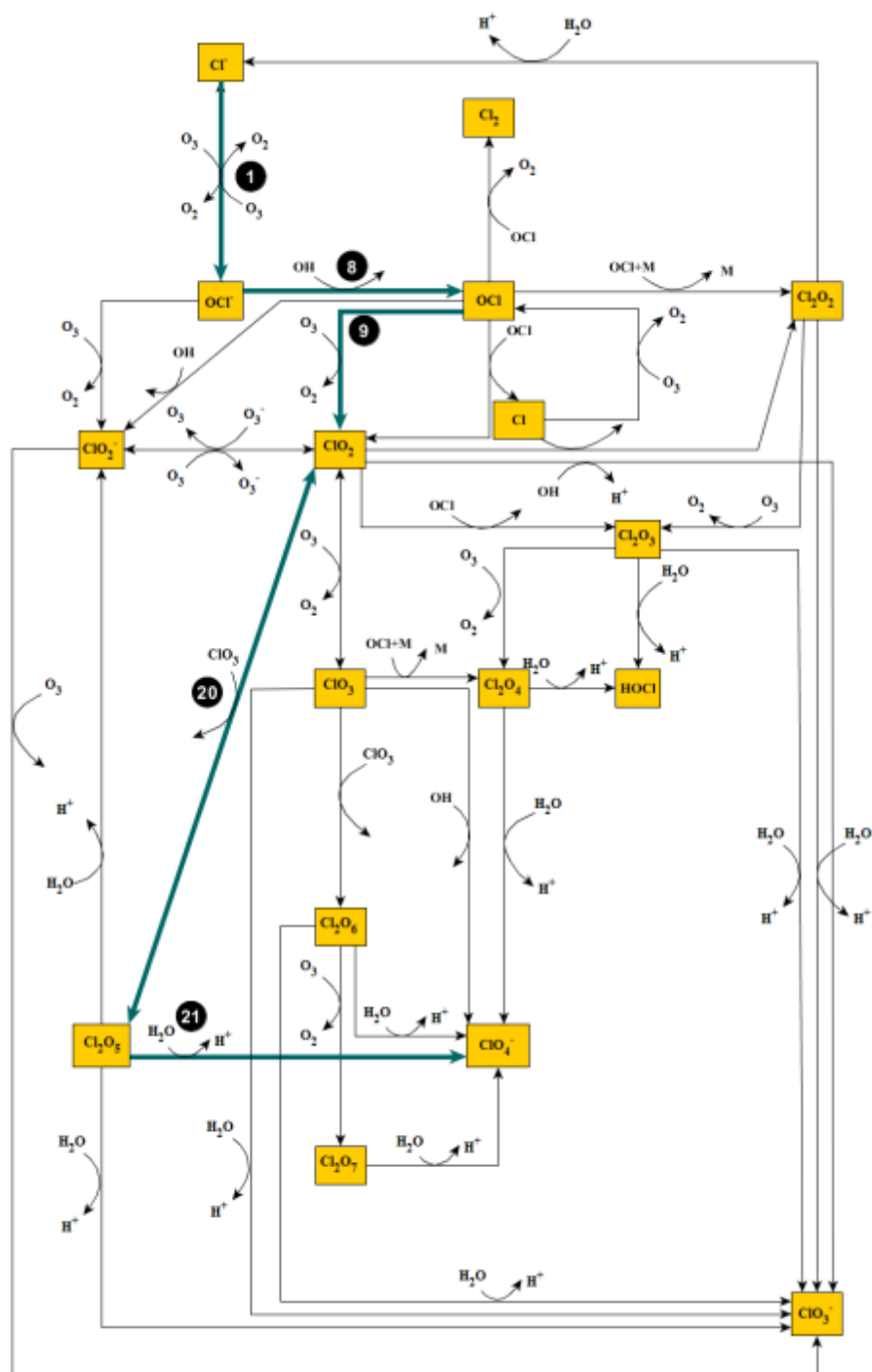


**Figure A.16 Mechanism 16.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq).** Note the thick dark cyan lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

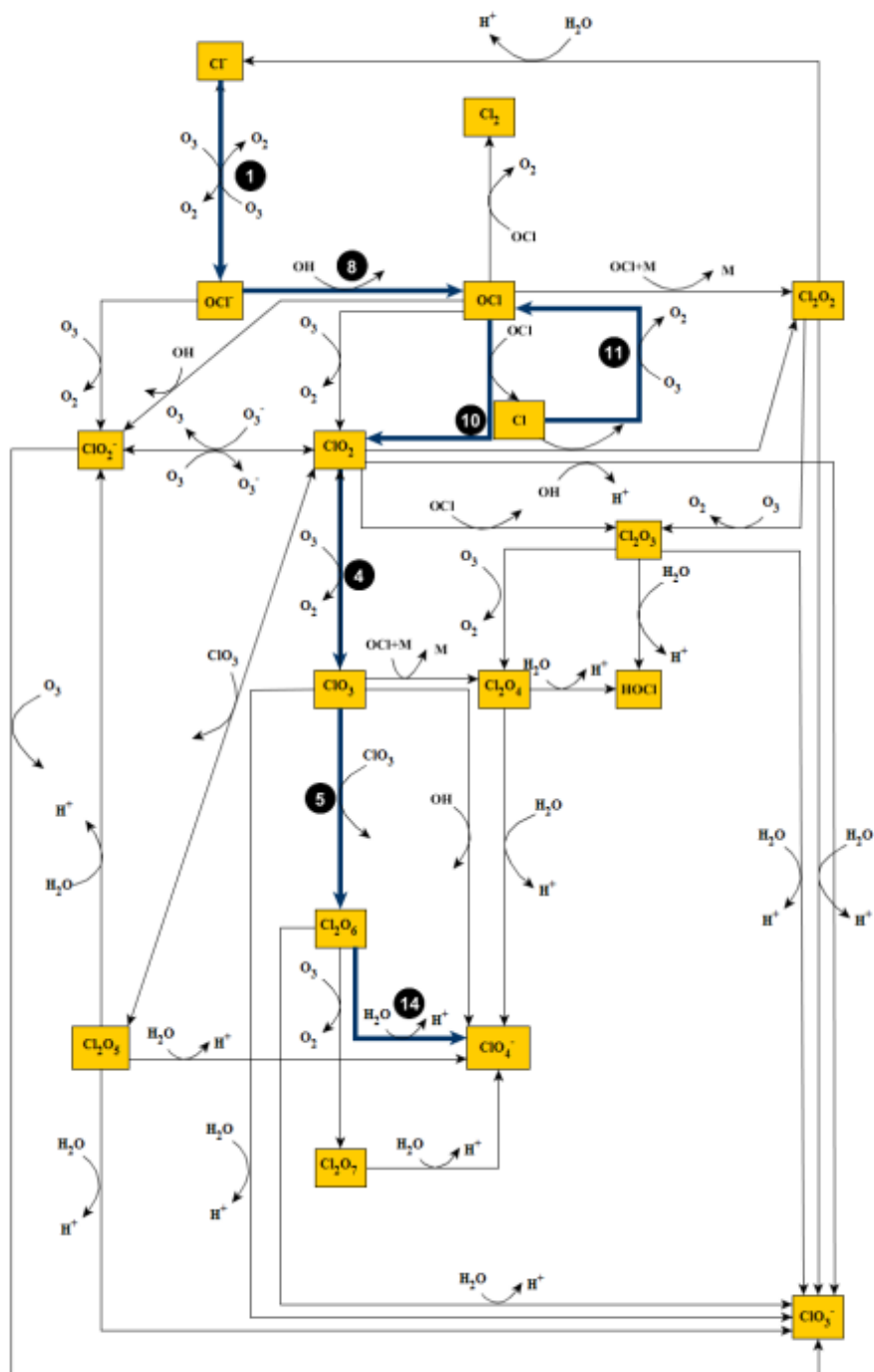


Figure A.17 Mechanism 17.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick dark blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$  and a  $\text{ClO}_3^-$  molecule containing all three O's from  $\text{O}_3$ .

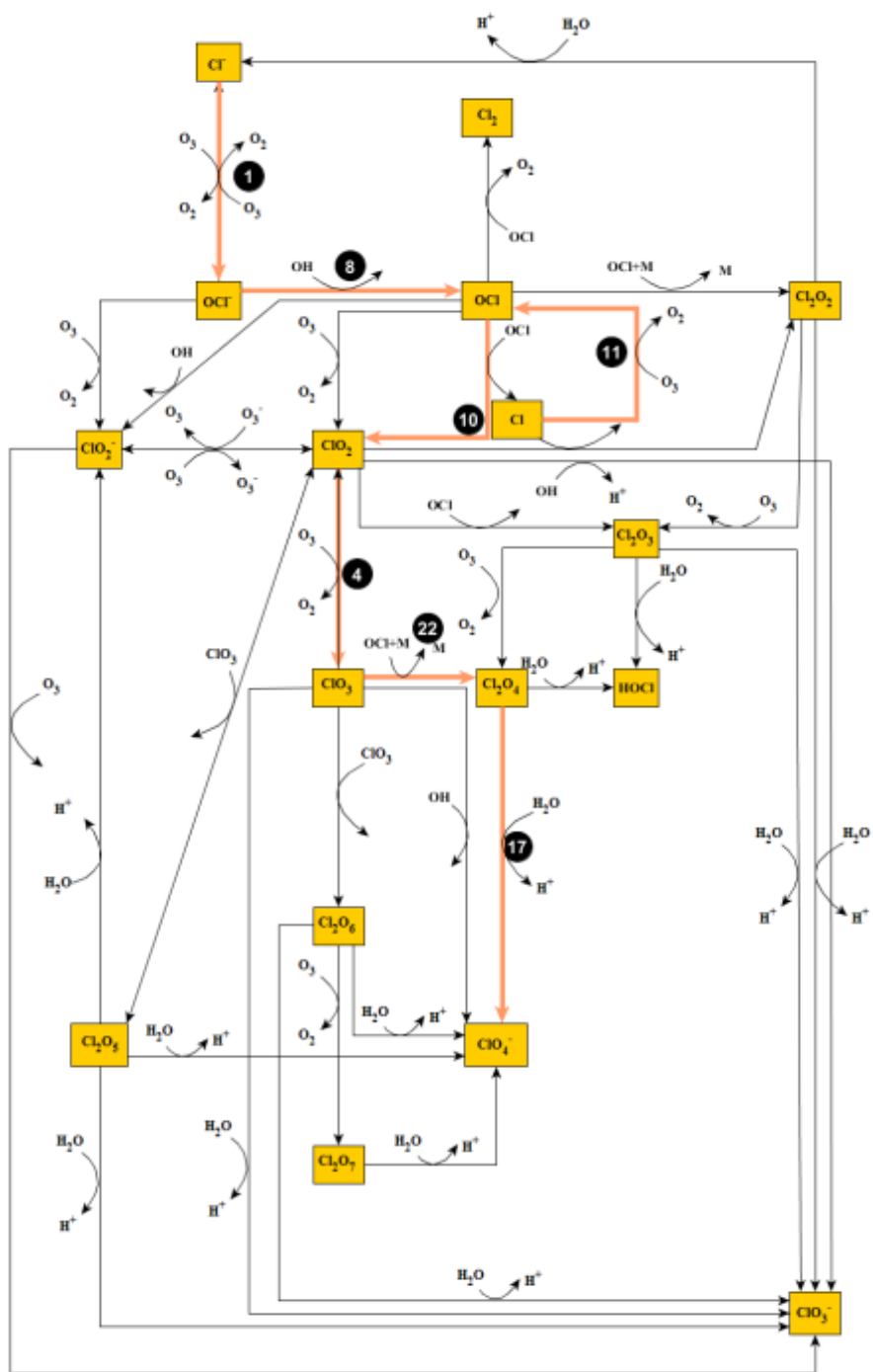


Figure A.18 Mechanism 18.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick apricot lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

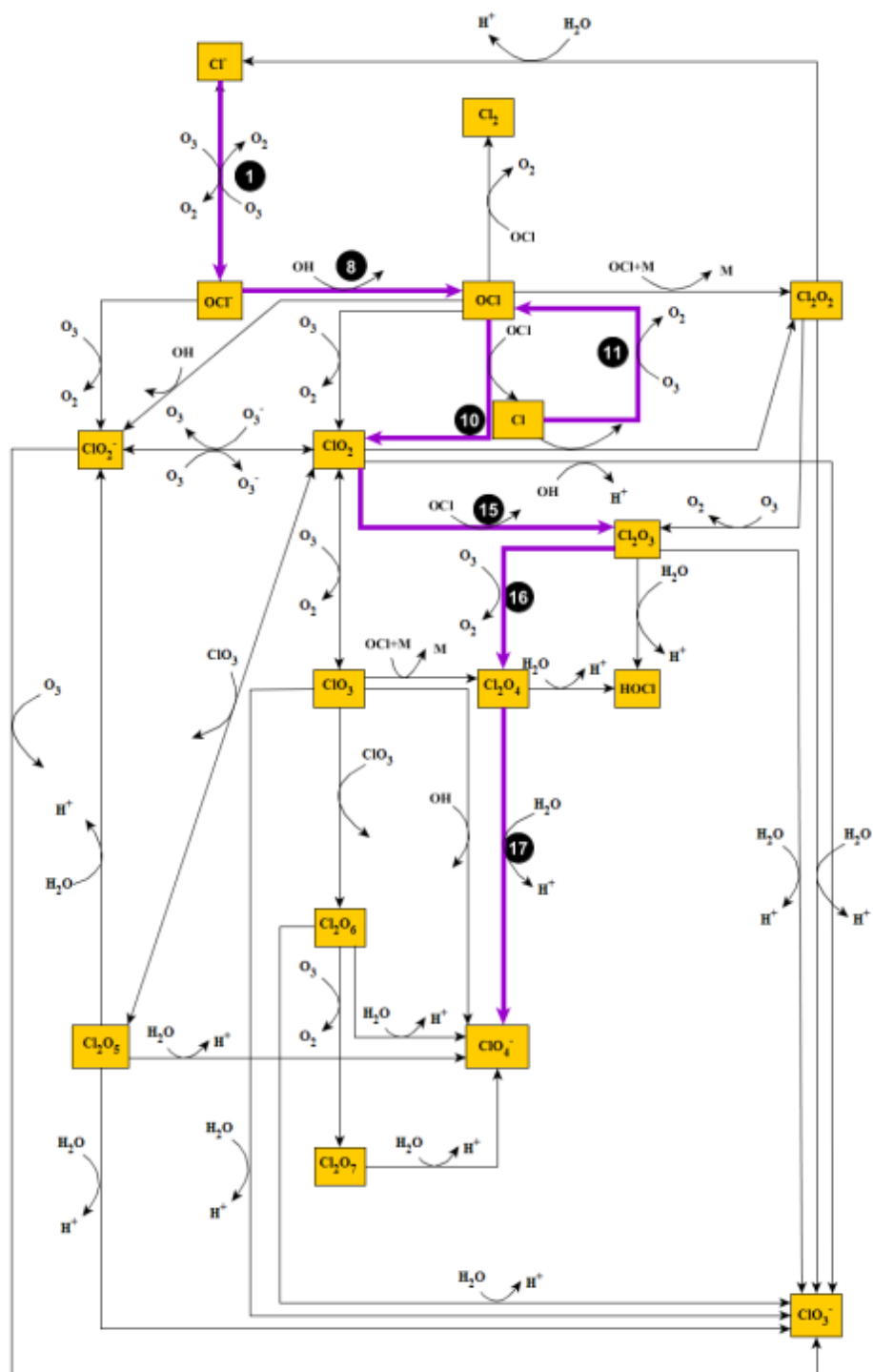


Figure A.19 Mechanism 19.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .



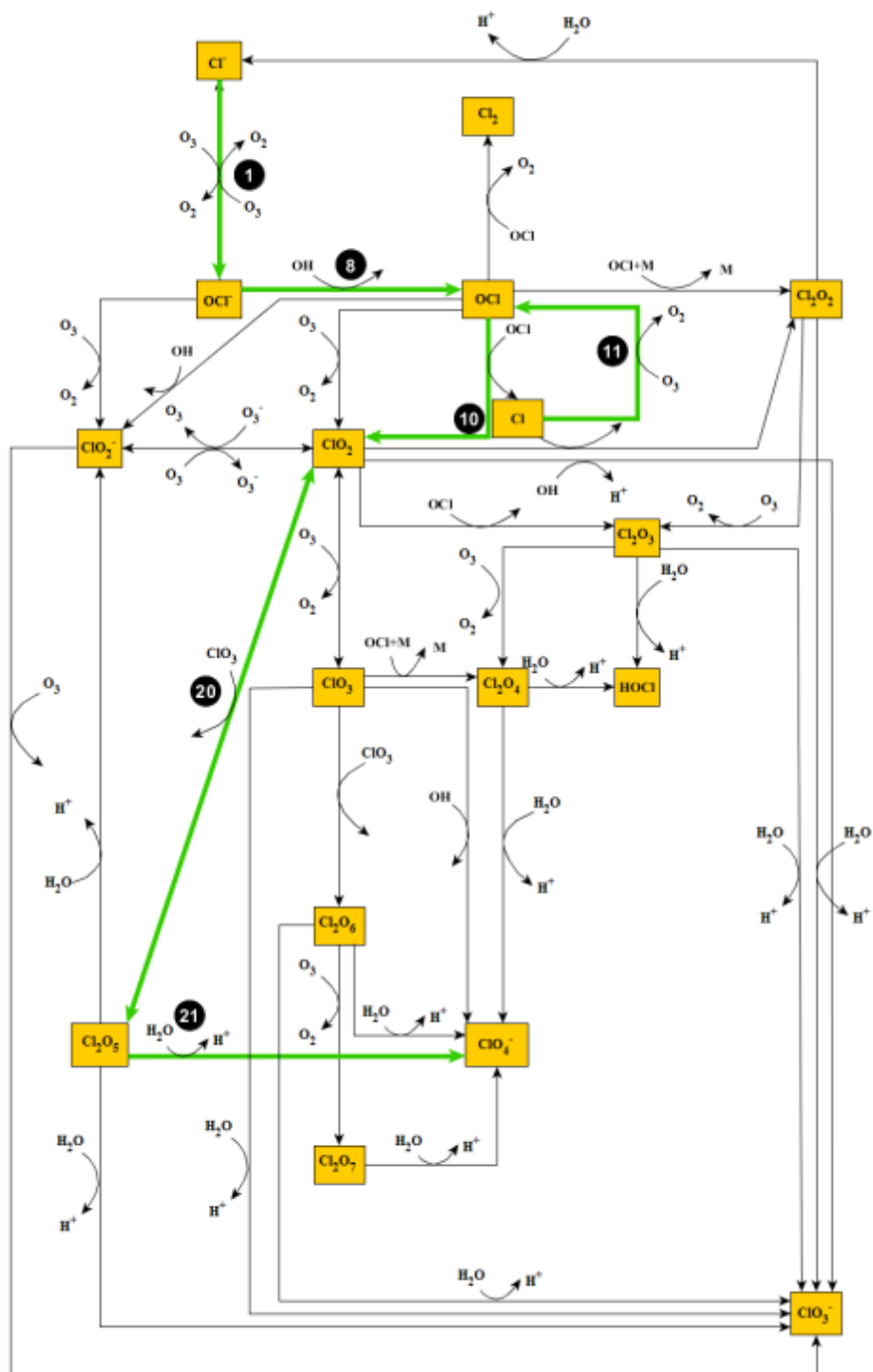


Figure A.20 Mechanism 20.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick lime green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

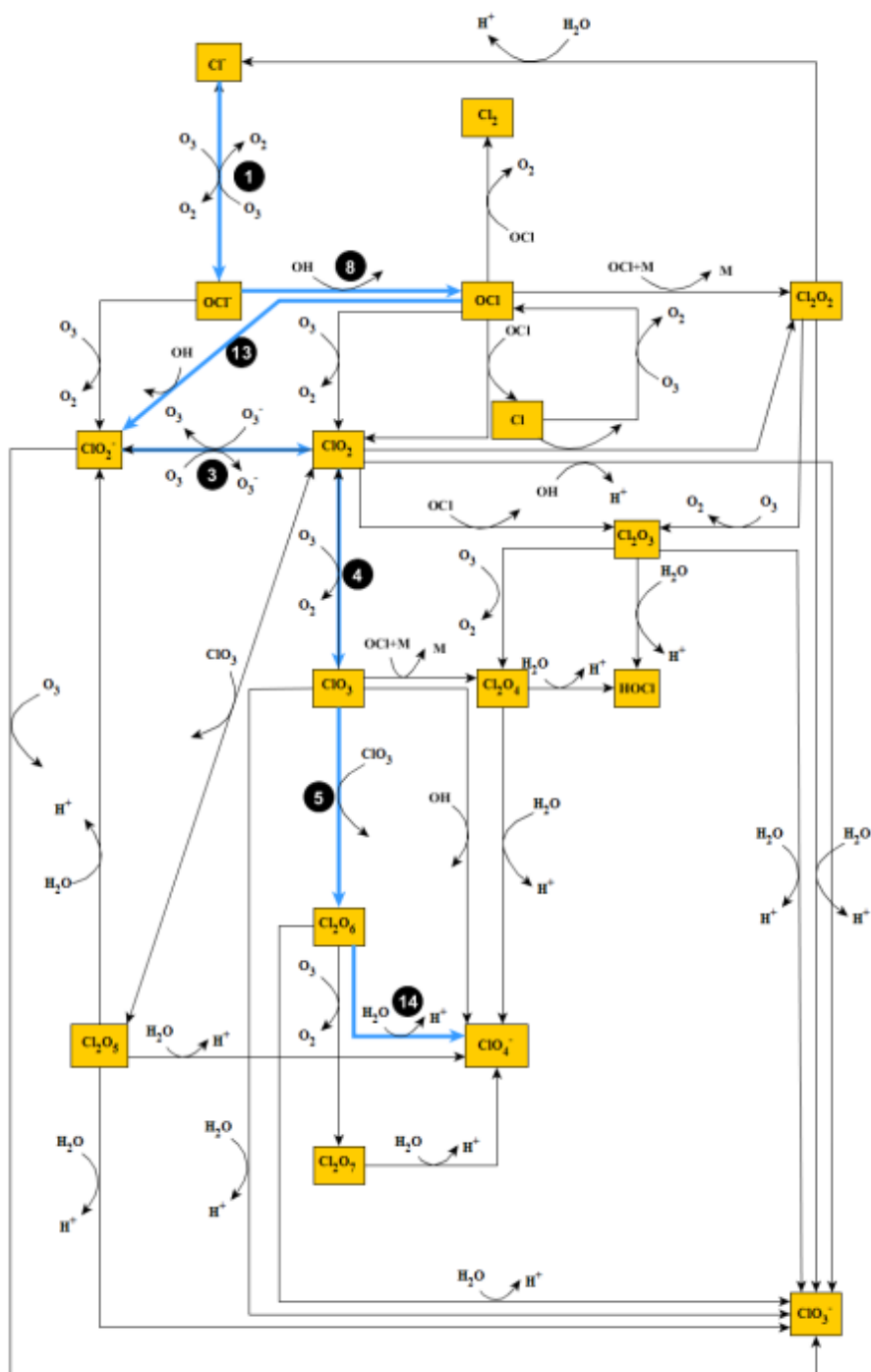


Figure A.21 Mechanism 21.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$  and a  $\text{ClO}_3^-$  molecule containing all three O's from  $\text{O}_3$ .

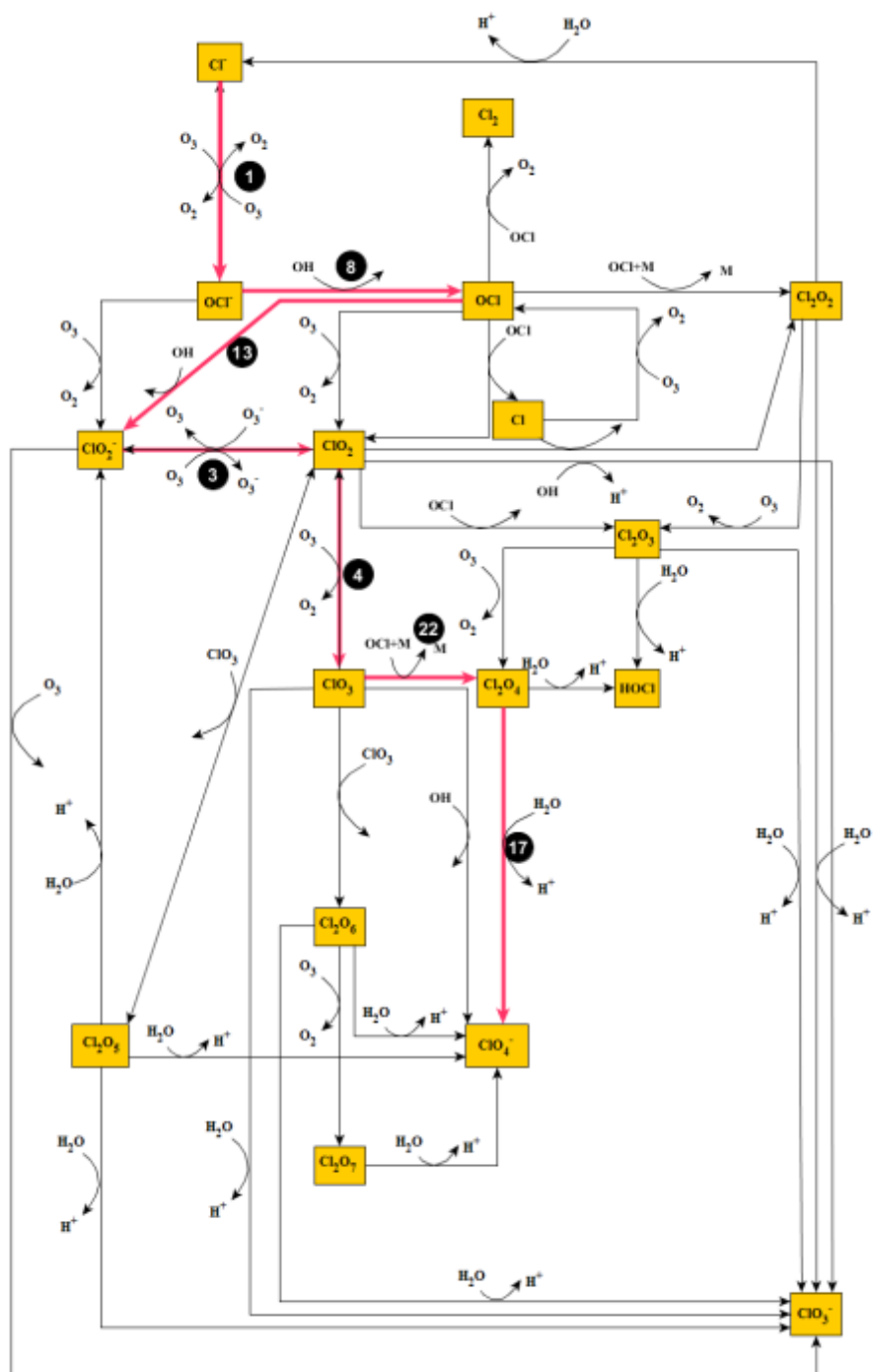


Figure A.22 Mechanism 22.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the pink lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

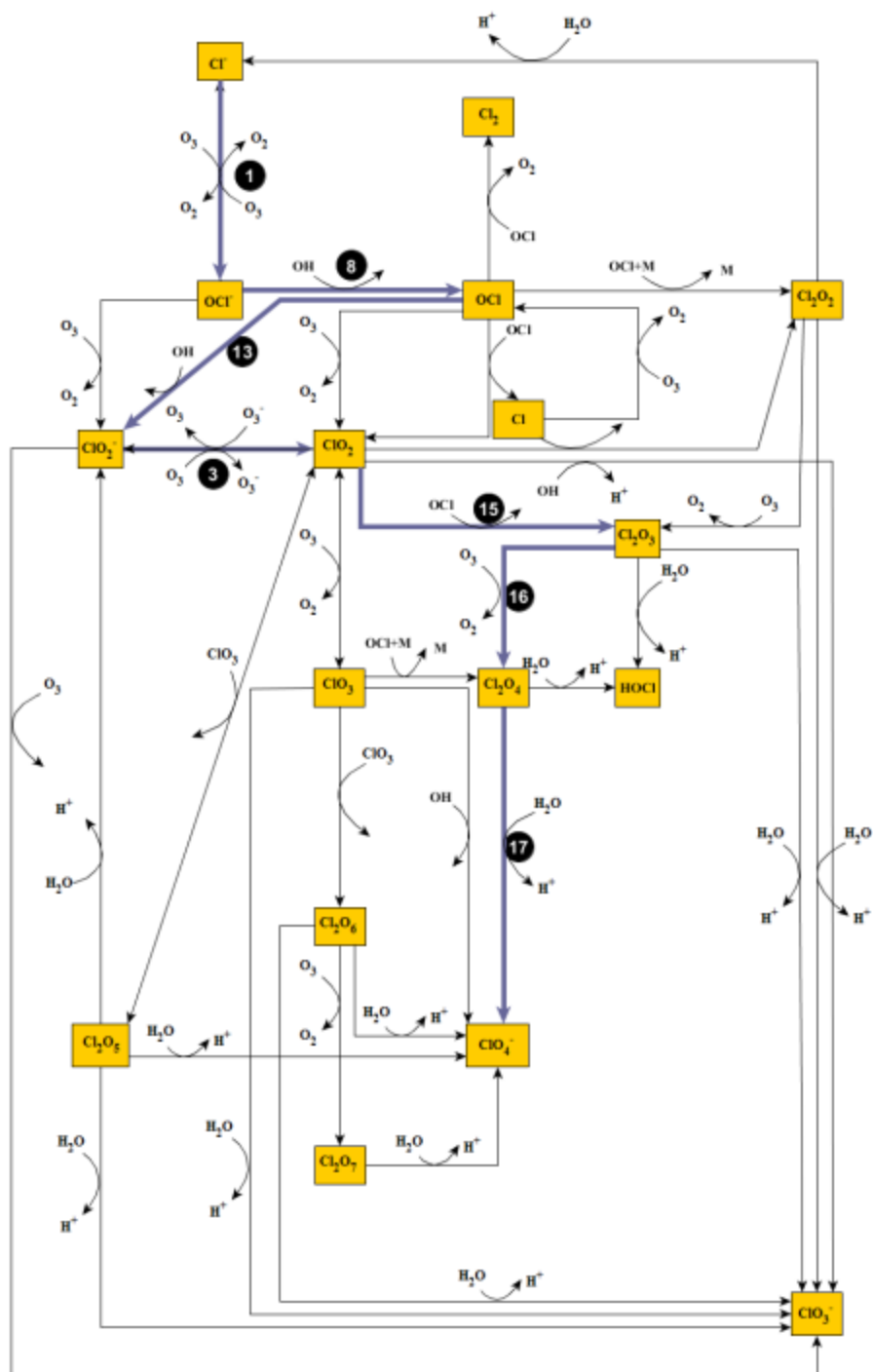


Figure A.23 Mechanism 23.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the grayish purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

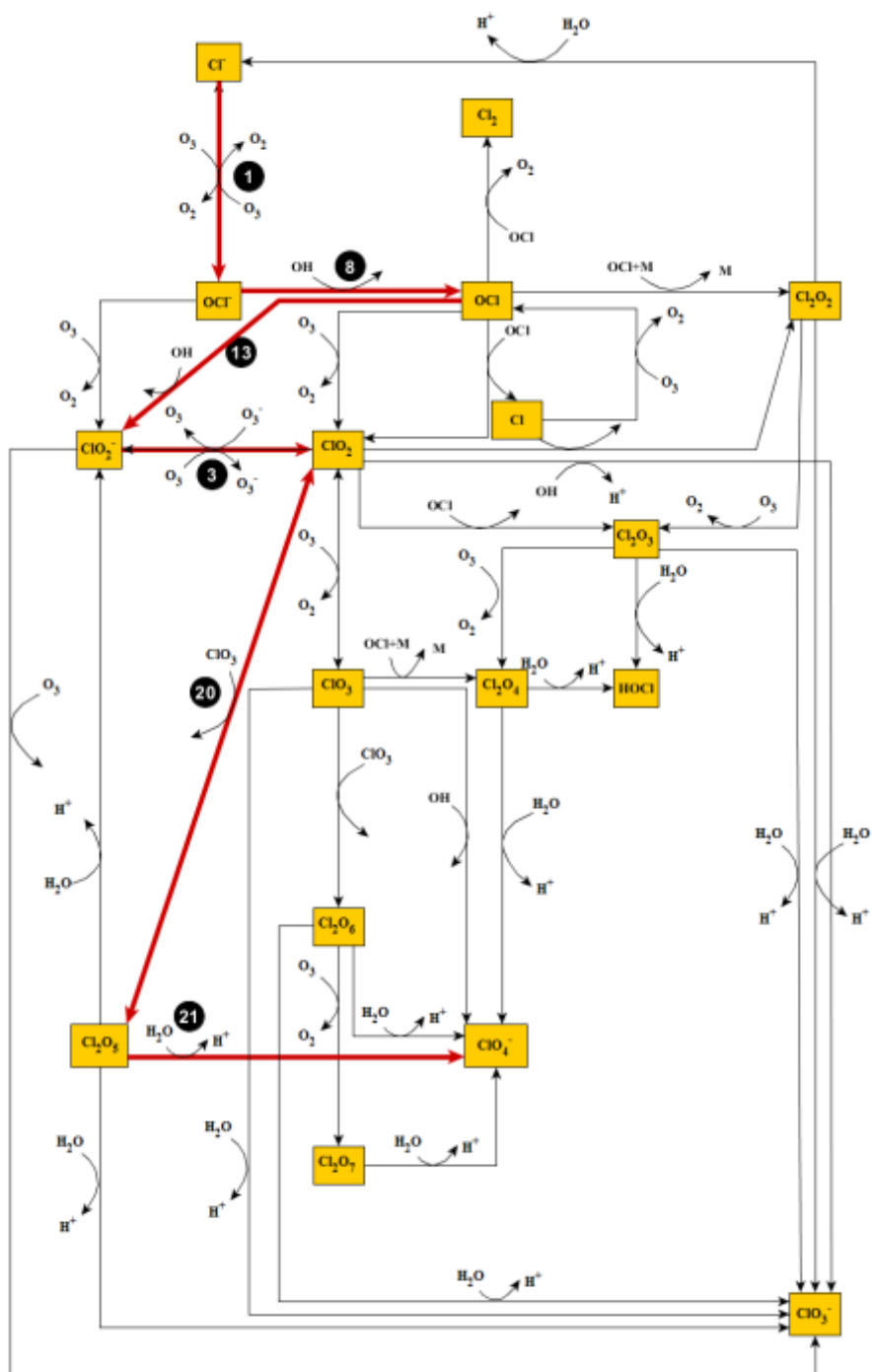


Figure A.24 Mechanism 24.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick red lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

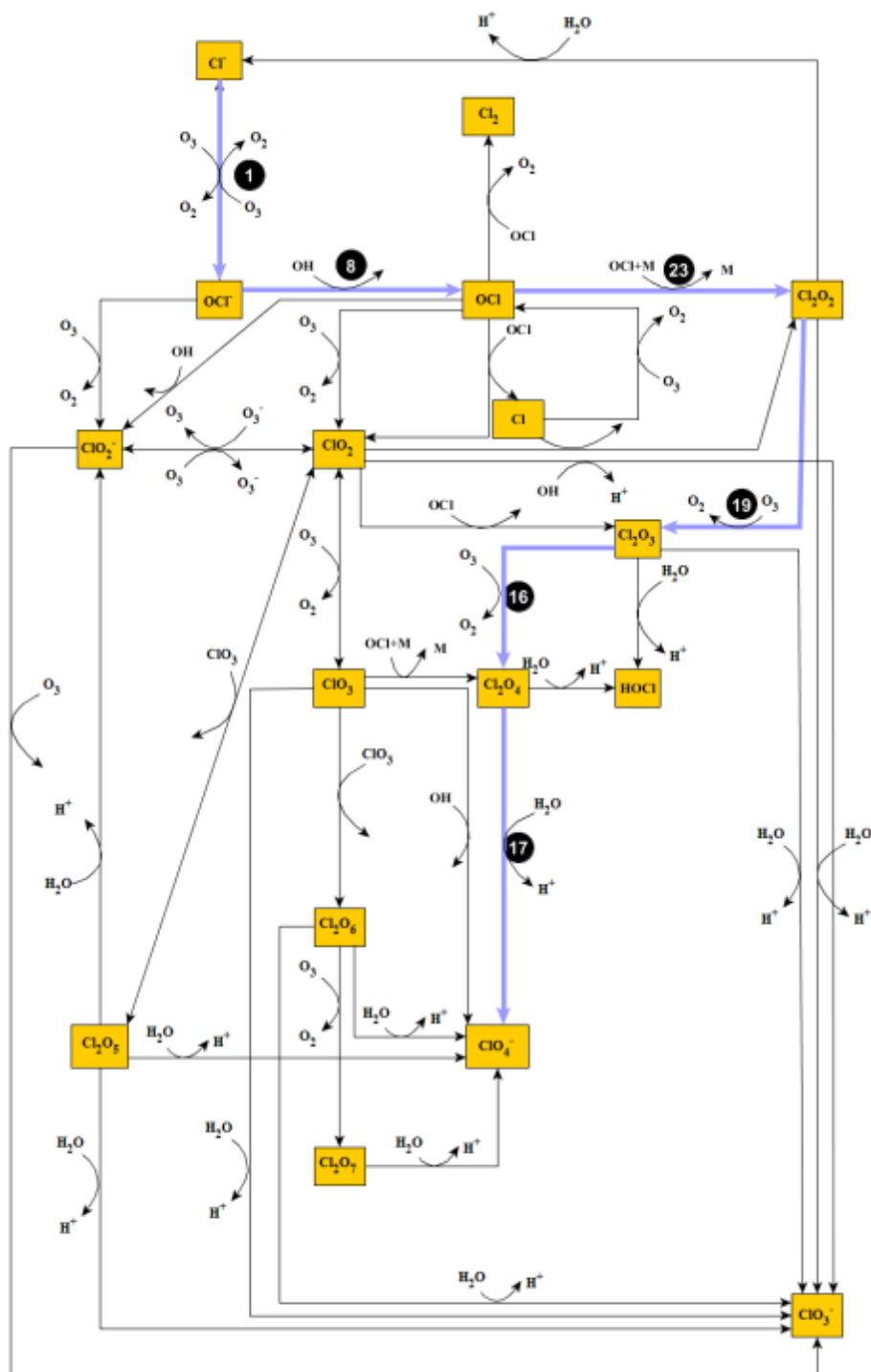


Figure A.25 Mechanism 25.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick red lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

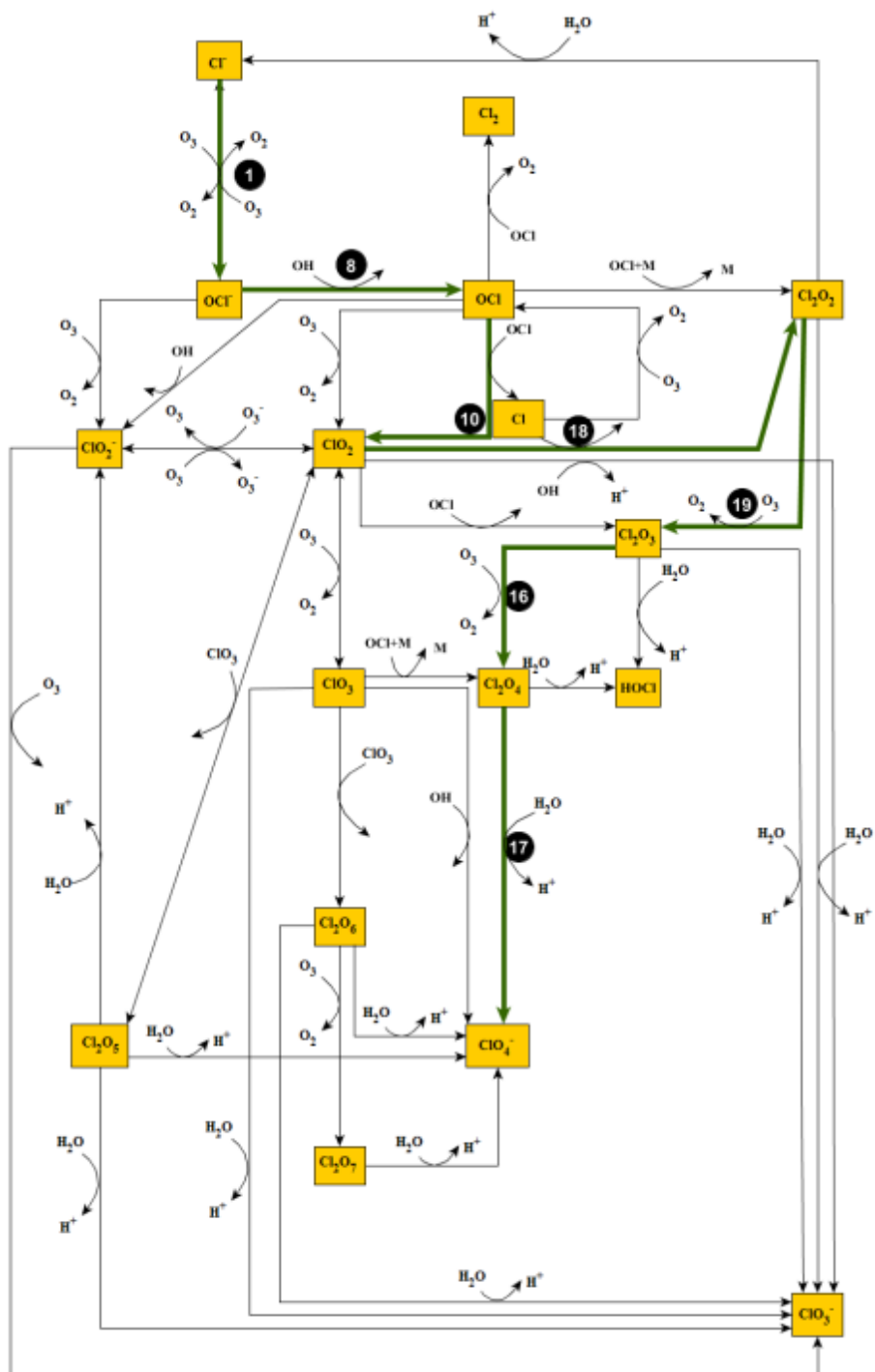


Figure A.26 Mechanism 26.  $\text{O}_3$  oxidation of  $\text{Cl}^-$  (aq). Note the thick forest green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

## A.2 Proposed Pathways for O<sub>3</sub> Oxidation of Hypochlorite (aq)

### A.2.1 Pathways that Produce ClO<sub>4</sub><sup>-</sup> with only three O's from O<sub>3</sub>

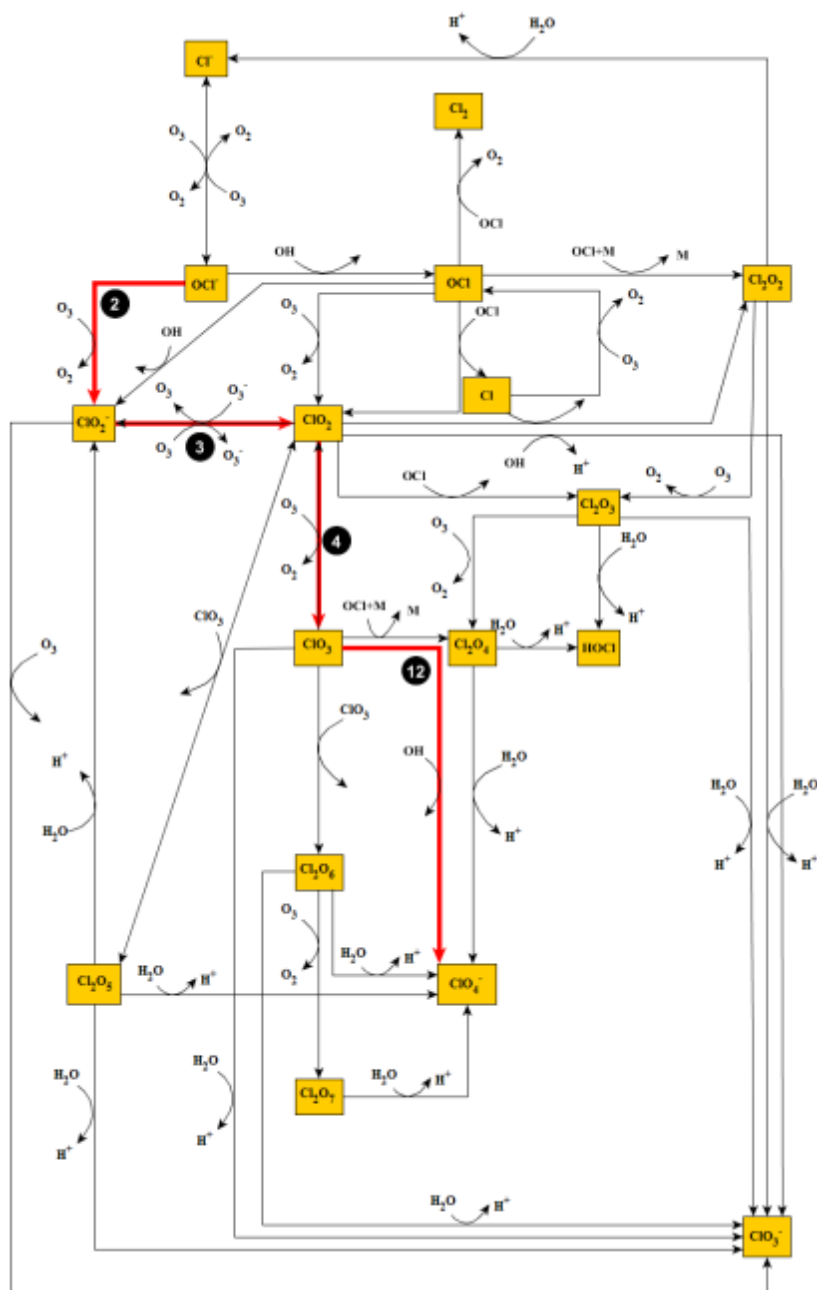


Figure A.27 Mechanism 1: O<sub>3</sub> oxidation of OCl<sup>-</sup> (aq). Note the thick red lines indicate the pathway leading to the formation of the ClO<sub>4</sub><sup>-</sup> and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO<sub>4</sub><sup>-</sup> molecule with three O's from O<sub>3</sub>.



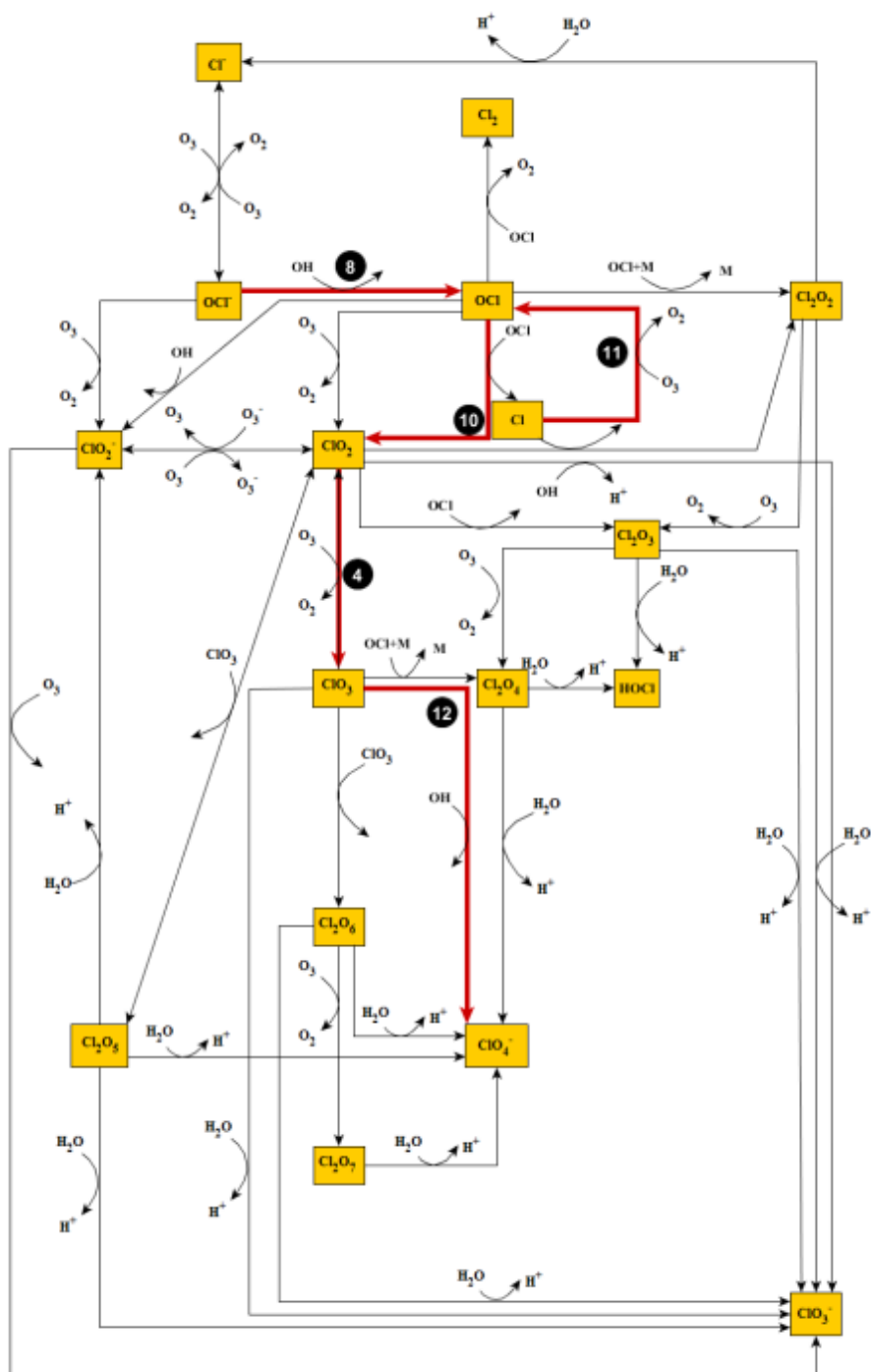


**Figure A.28 Mechanism 2:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq).** Note the thick red lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

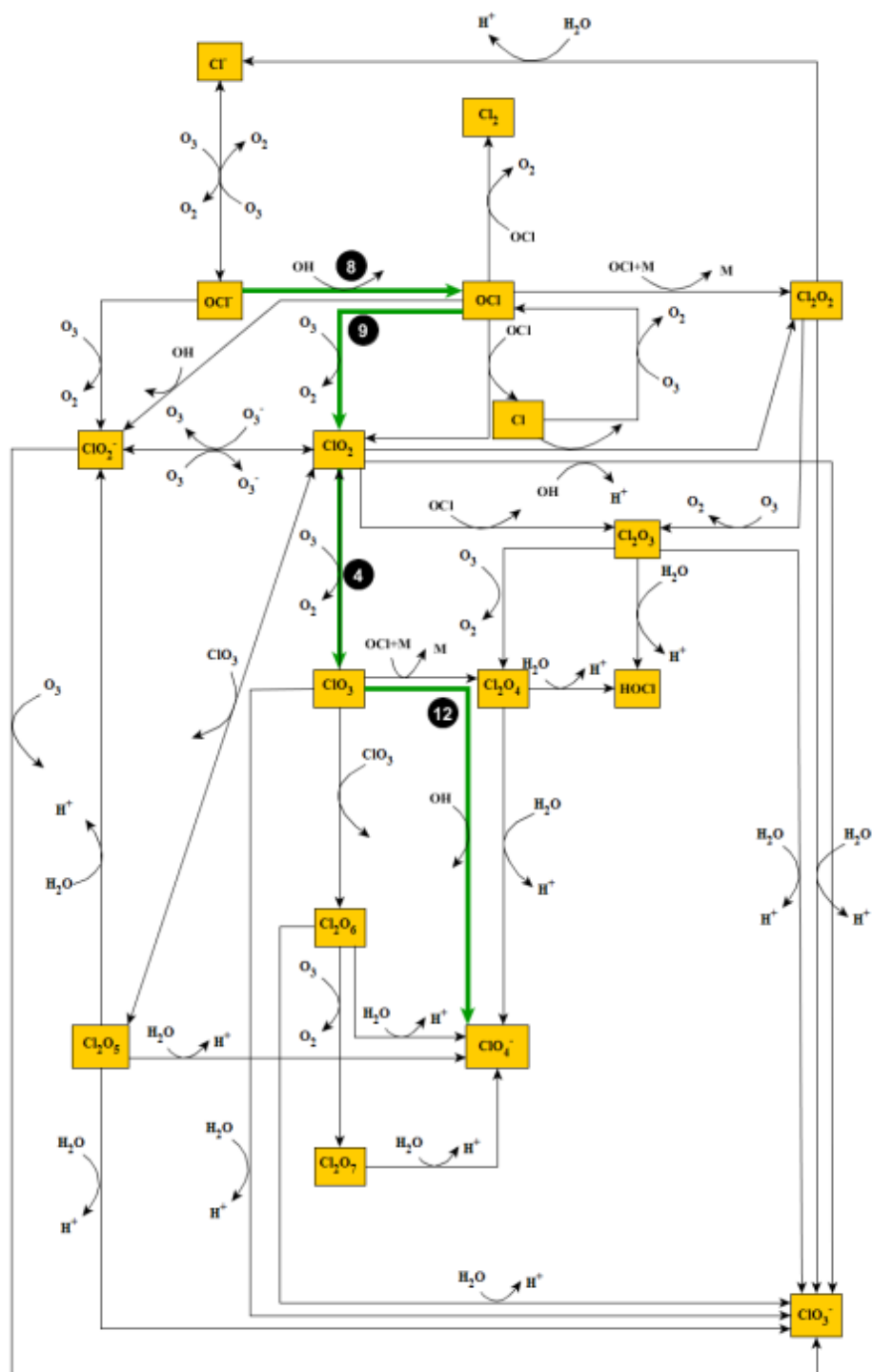


**Figure A.29 Mechanism 3:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq).** Note the thick green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

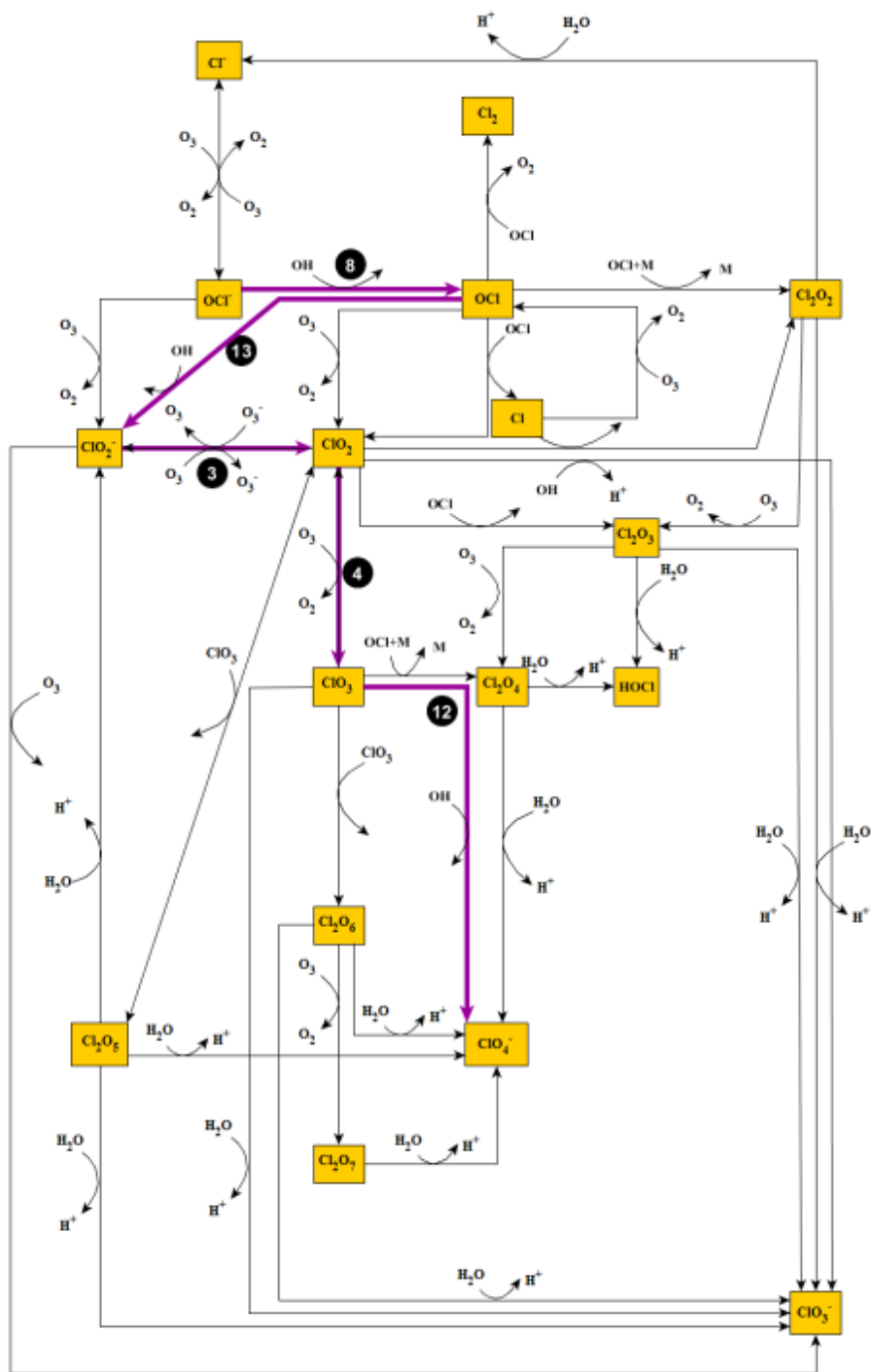


Figure A.30 Mechanism 4:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing three O's from  $\text{O}_3$ .

### A.2.1 Pathways that Produce $\text{ClO}_4^-$ with two and three O's from $\text{O}_3$

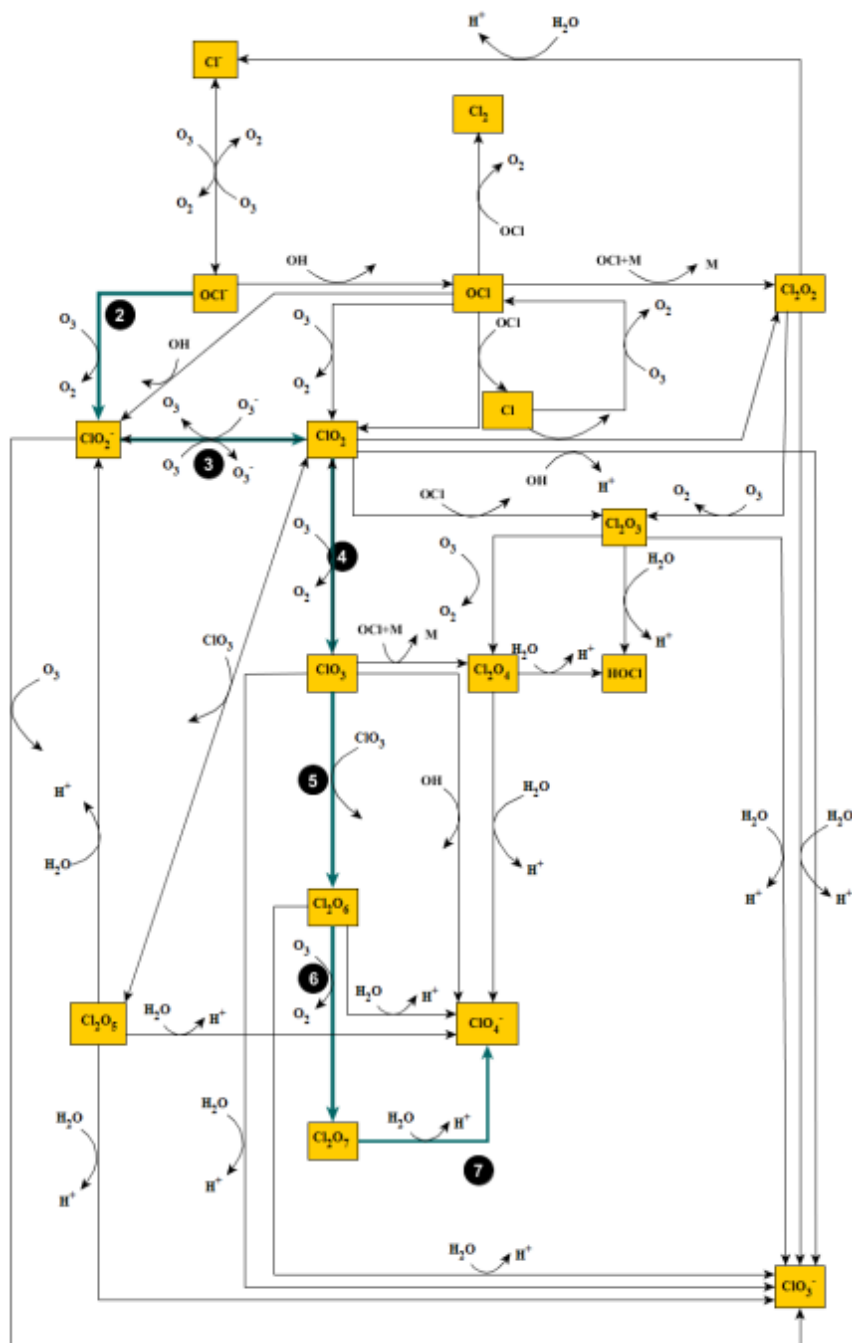


Figure A.31 Mechanism 5:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick dark aqua lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$  molecules, one with three O from  $\text{O}_3$  and the other with two O from  $\text{O}_3$ .

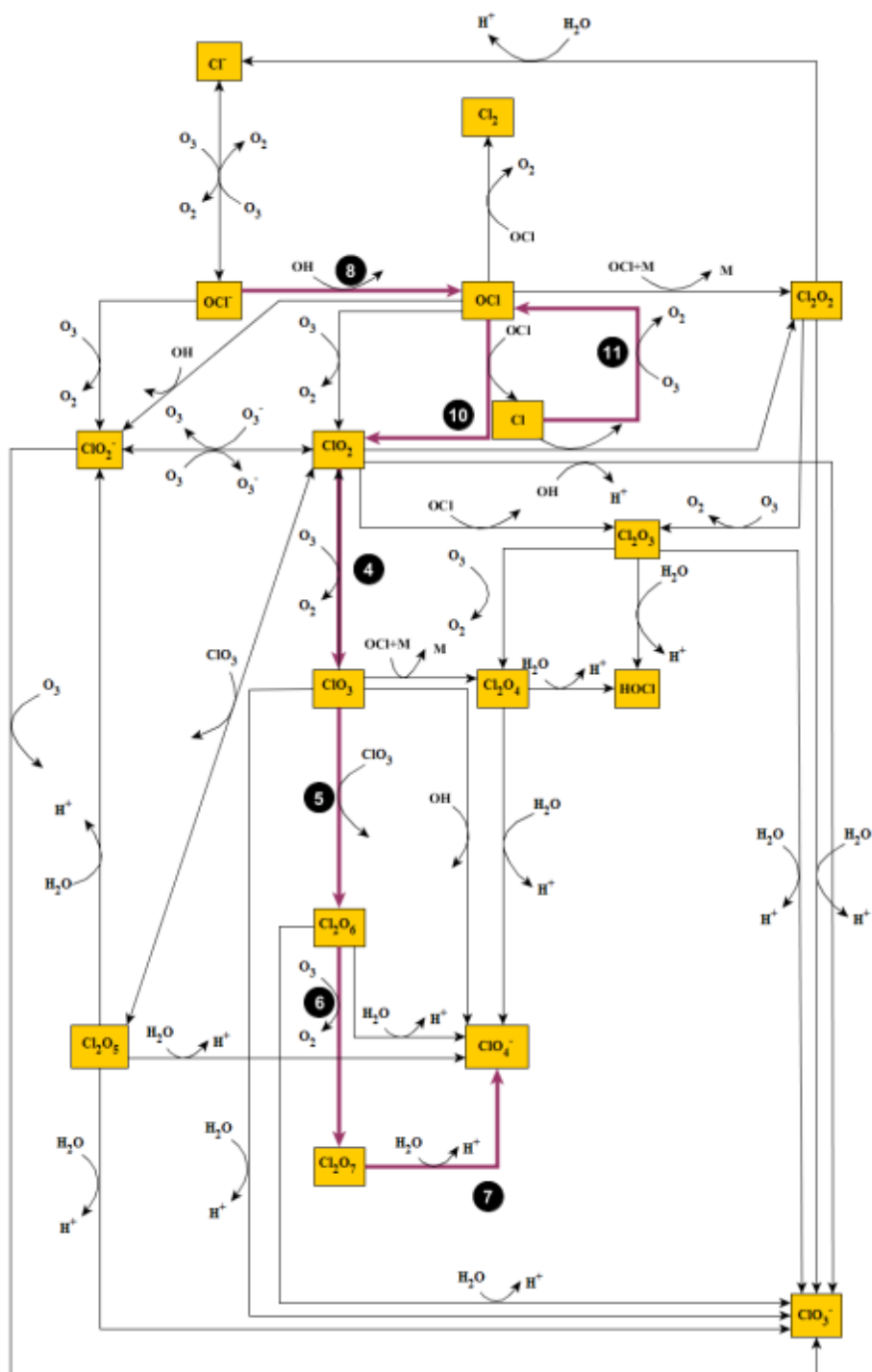


Figure A.32 Mechanism 6:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick dark pink lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$  molecules, one with three O from  $\text{O}_3$  and the other with two O from  $\text{O}_3$ .

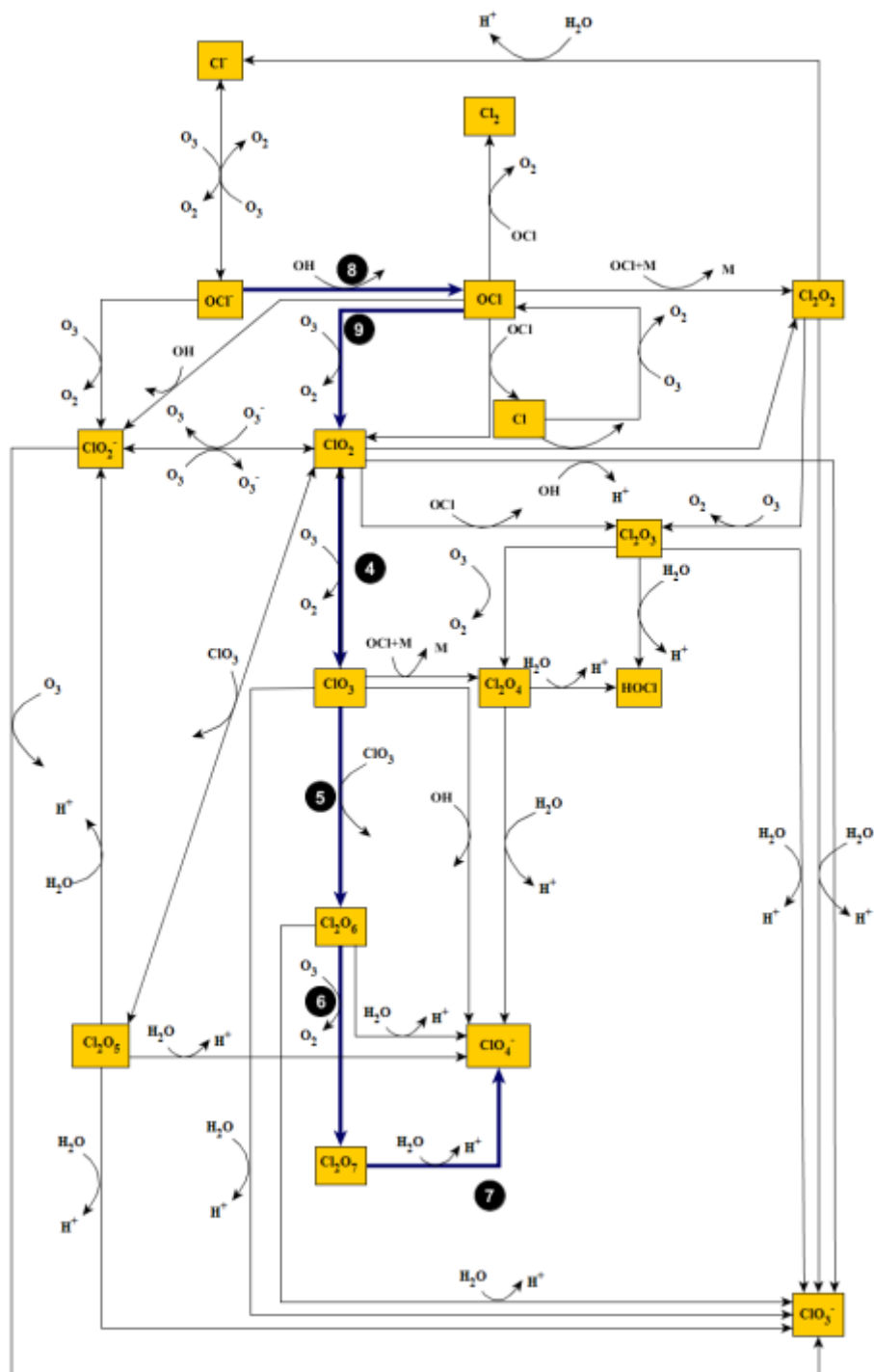


Figure A.33 Mechanism 7:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick dark purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$  molecules, one with three O from  $\text{O}_3$  and the other with two O from  $\text{O}_3$ .

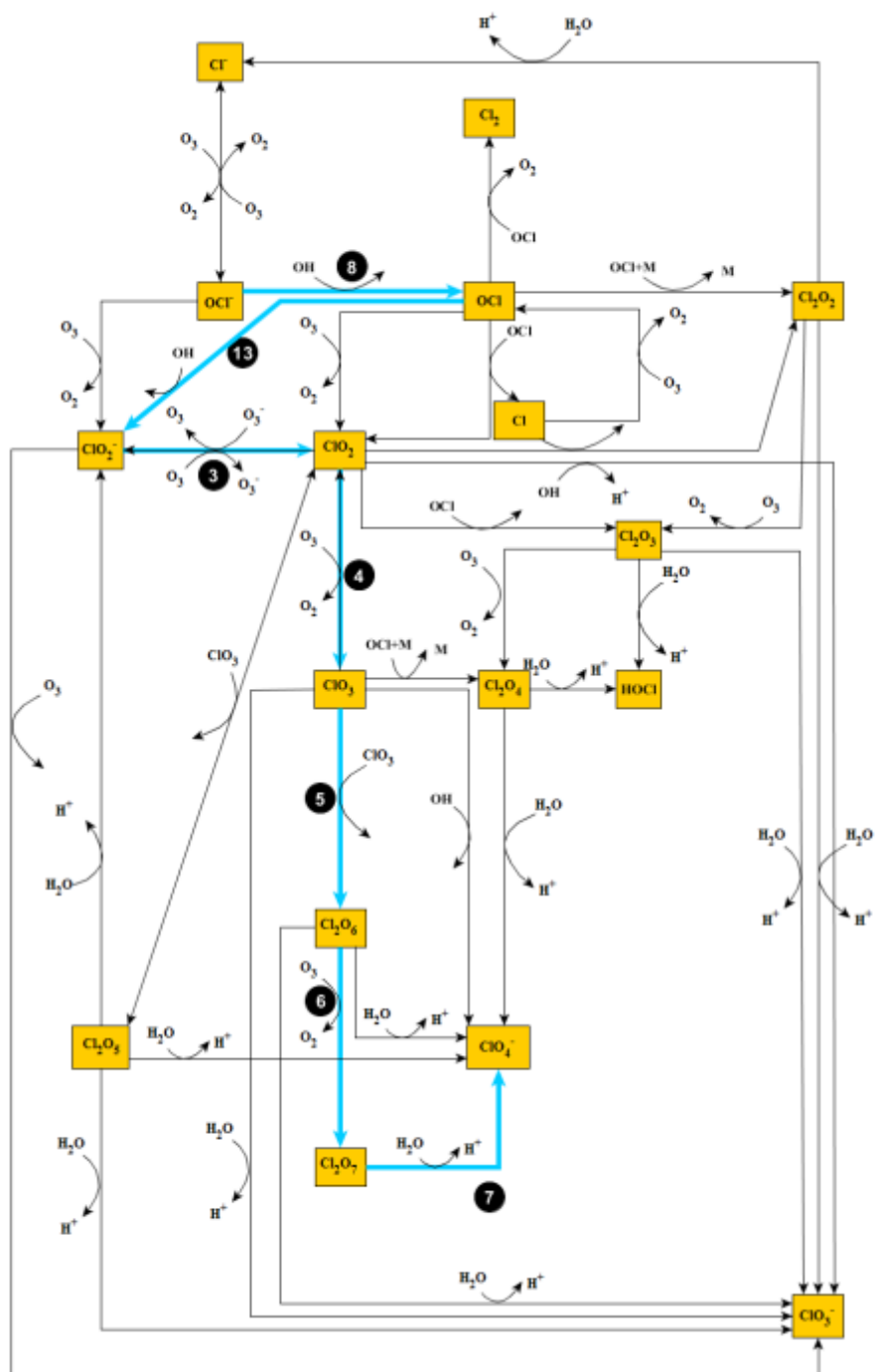


**Figure A.34 Mechanism 8:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq).** Note the thick blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$  molecules, one with three O from  $\text{O}_3$  and the other with two O from  $\text{O}_3$ .

### A.2.2 Pathways that Produce $\text{ClO}_4^-$ with only two O's from $\text{O}_3$

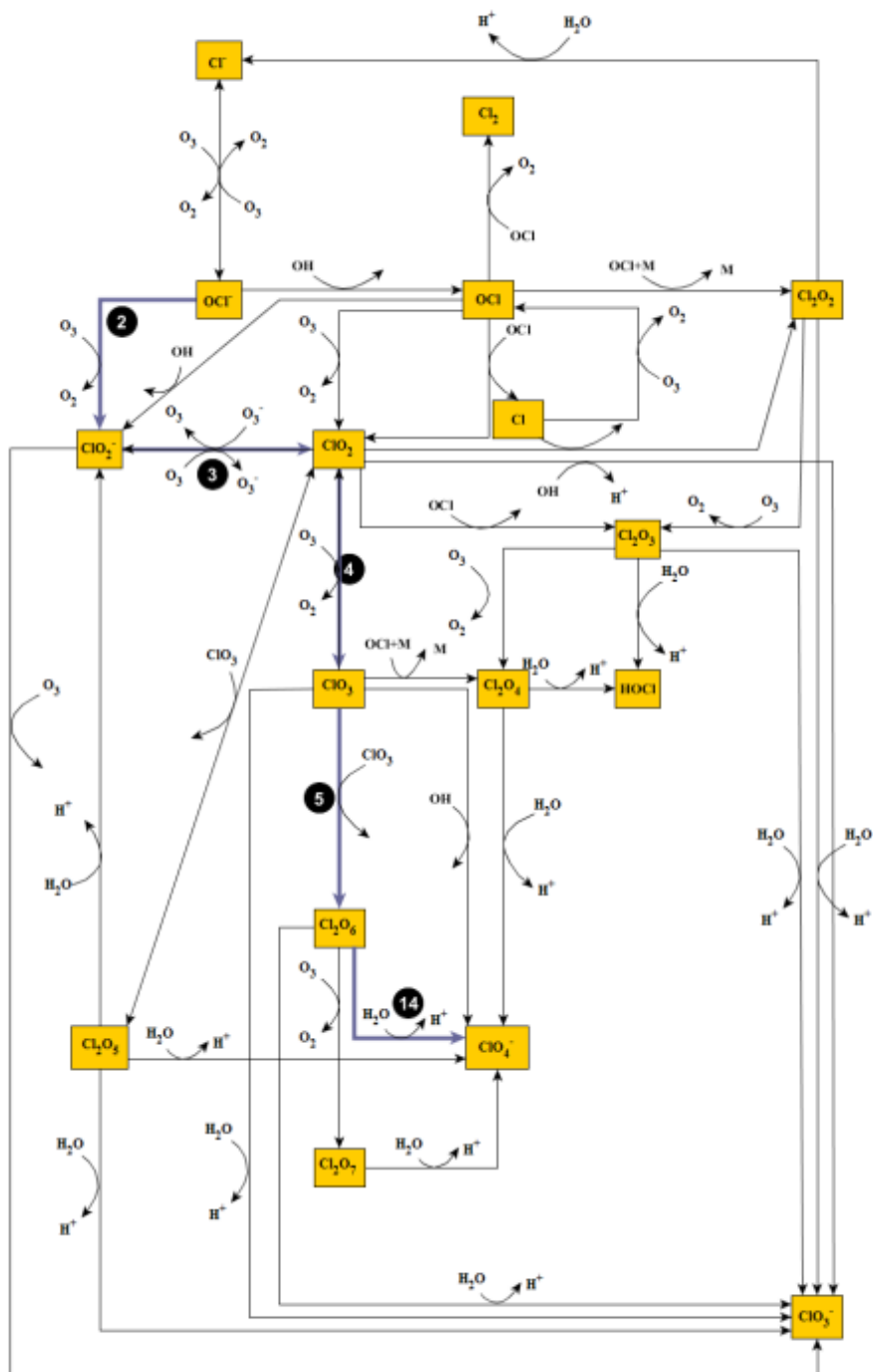


**Figure A.35 Mechanism 9: O<sub>3</sub> oxidation of OCl<sup>-</sup> (aq). Note the thick purple lines indicate the pathway leading to the formation of the ClO<sub>4</sub><sup>-</sup> and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one ClO<sub>4</sub><sup>-</sup> molecule containing two O's from O<sub>3</sub>.**



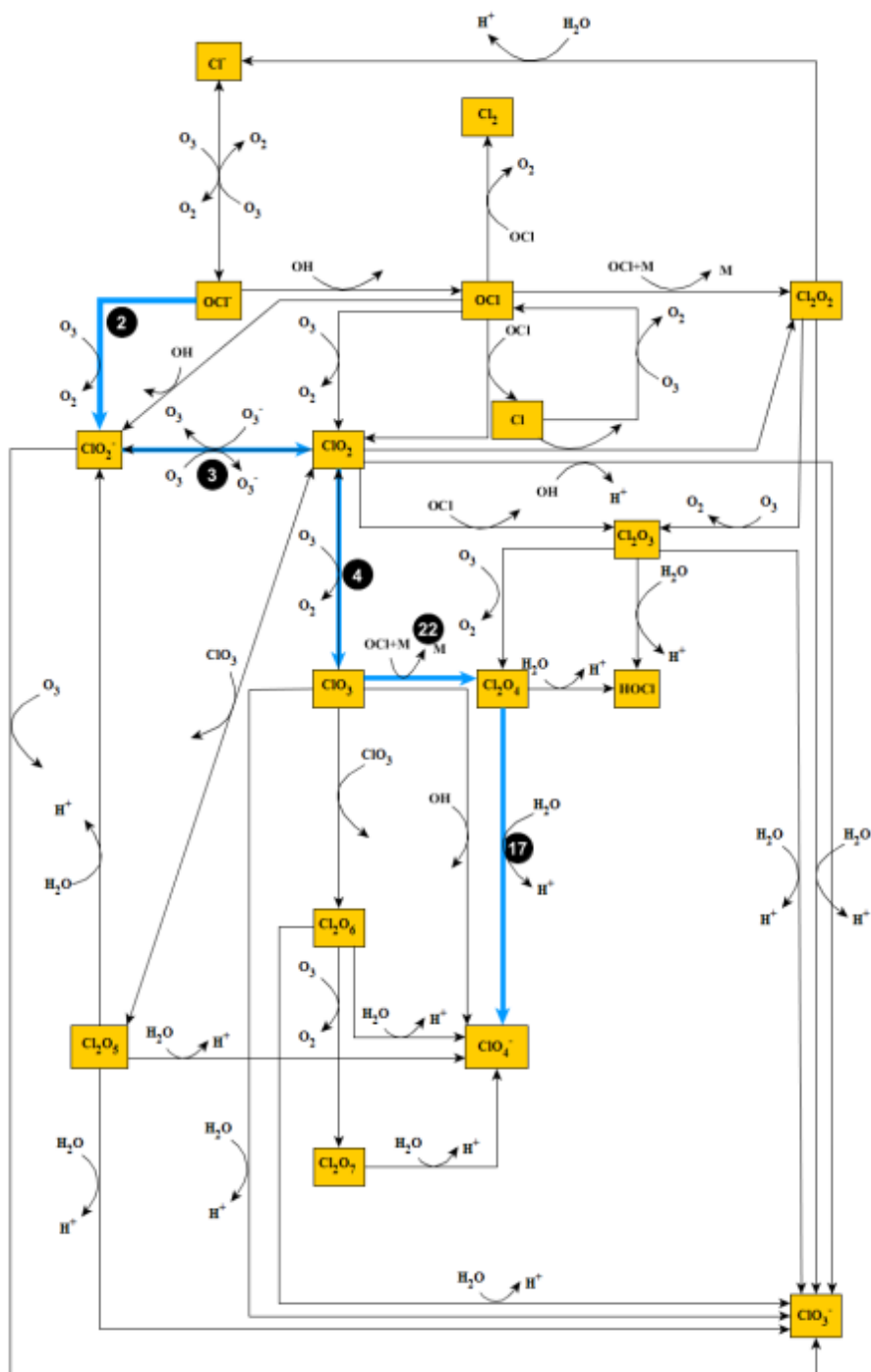


Figure A.36 Mechanism 10:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

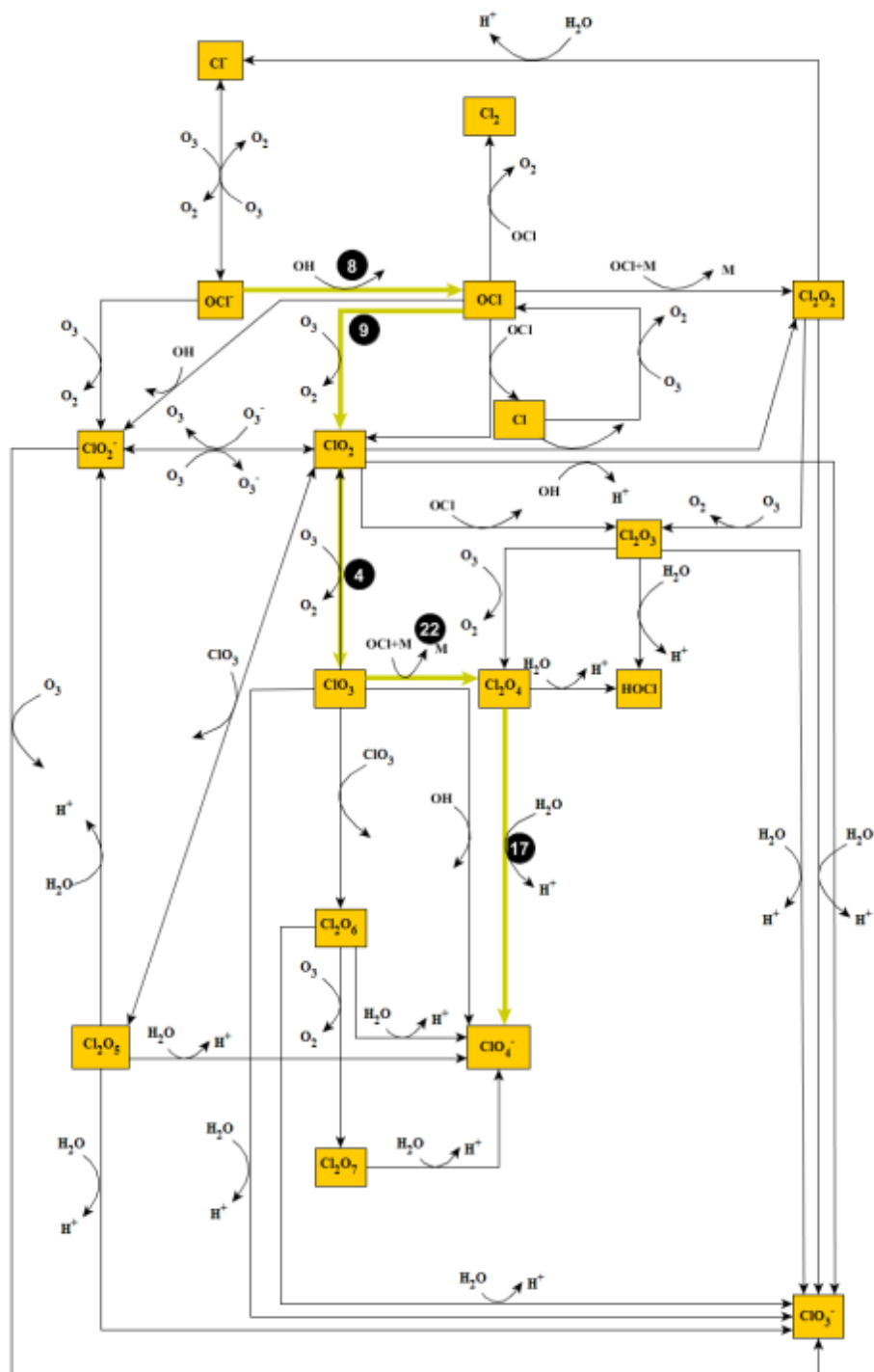


**Figure A.37 Mechanism 11:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq).** Note the thick yellow lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

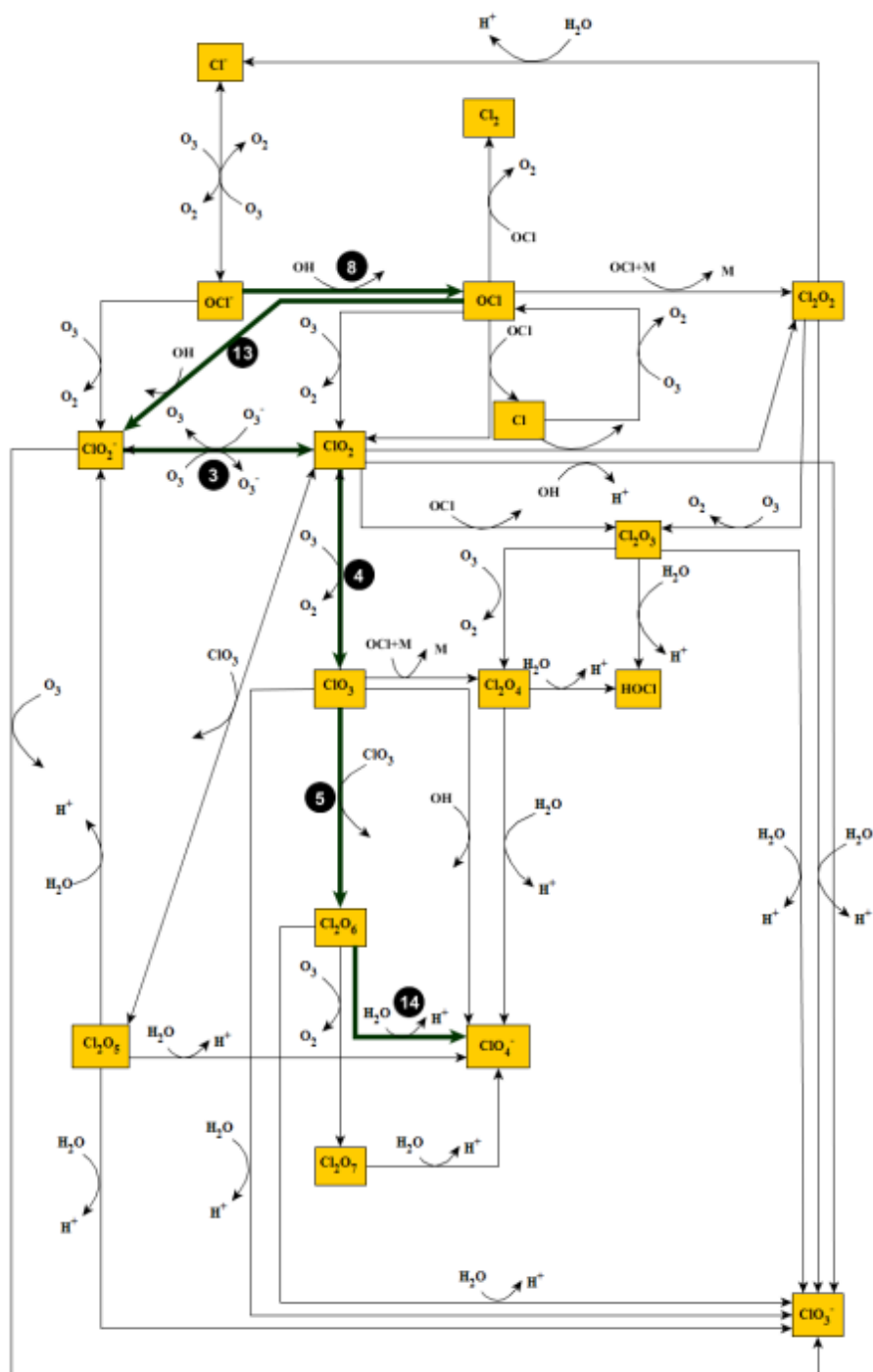


Figure A.38 Mechanism 12:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick dark green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

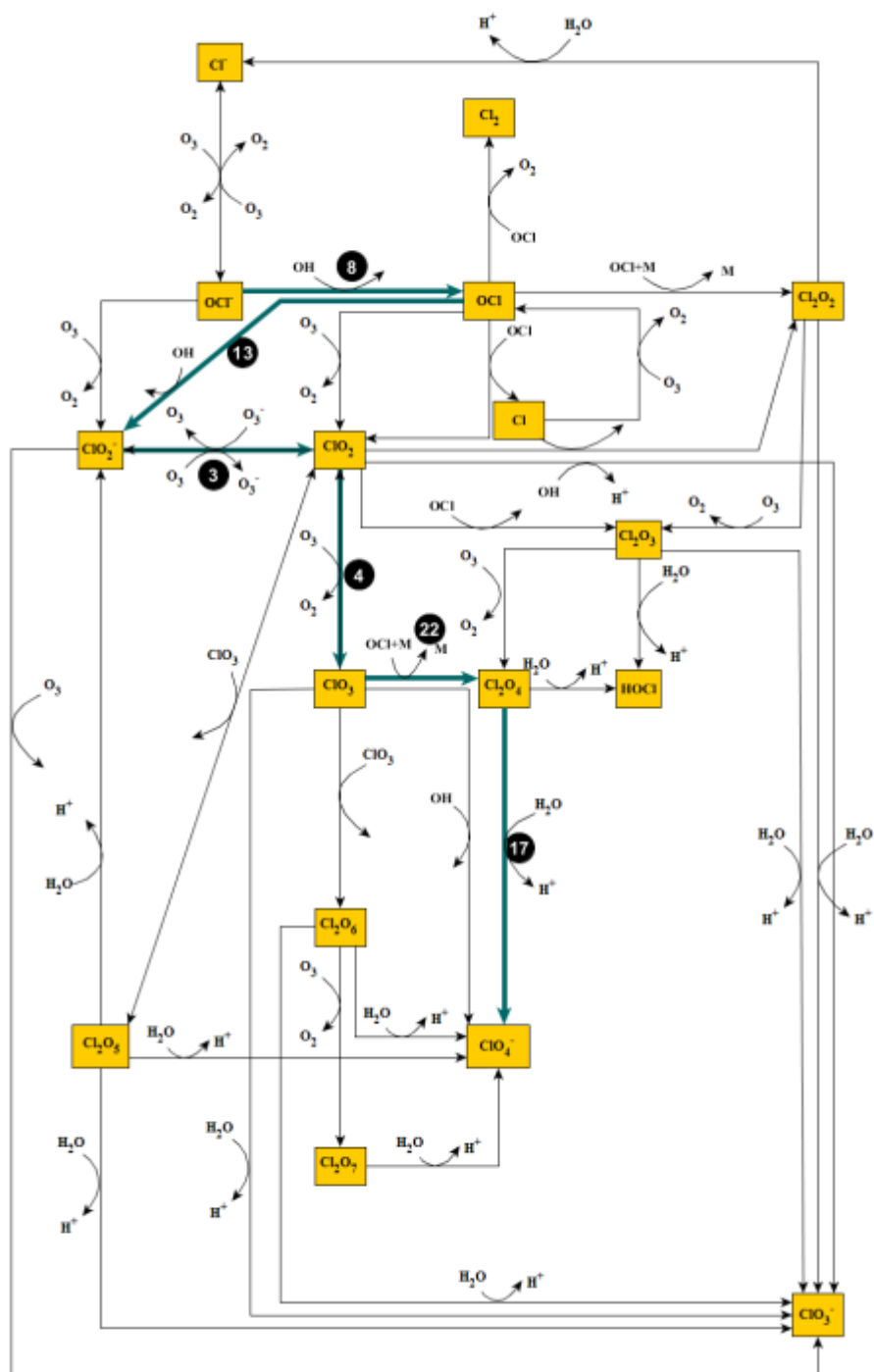


Figure A.39 Mechanism 13:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick aqua green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

### A.2.3. Pathways involving the $\text{Cl}_2\text{O}_5$ Species

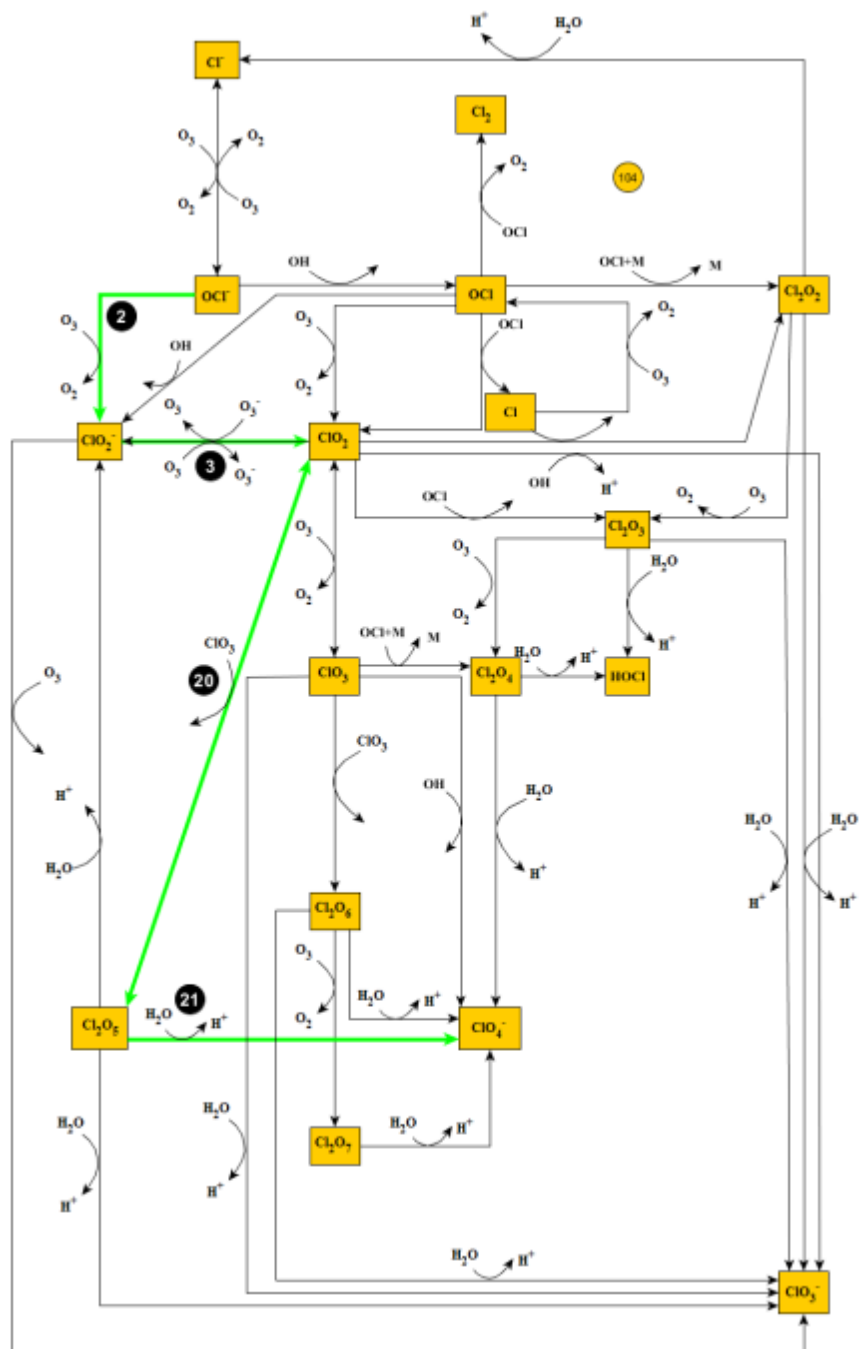


Figure A.40 Mechanism 14:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick neon green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

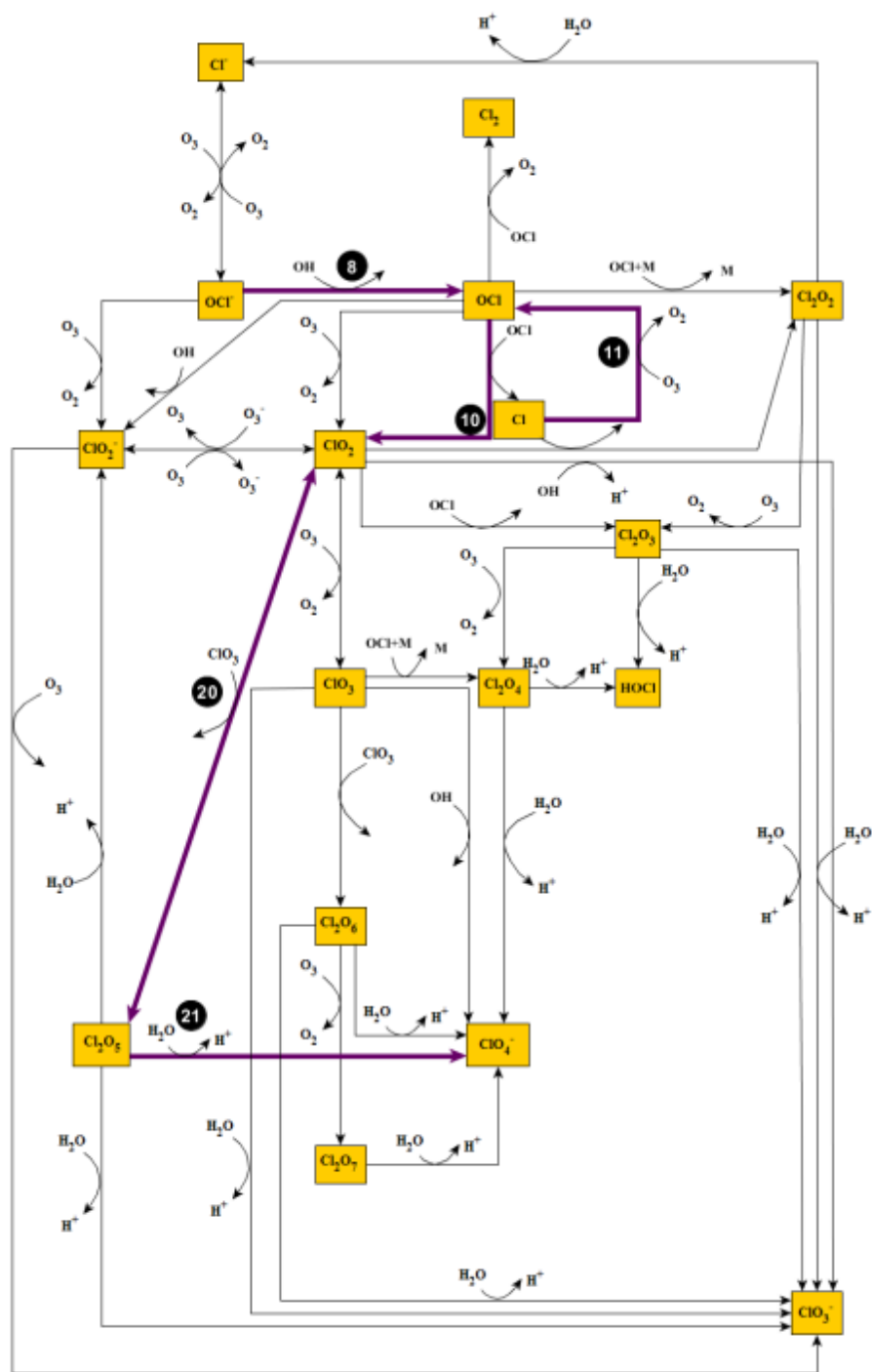


**Figure A.41 Mechanism 15:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq).** Note the thick purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

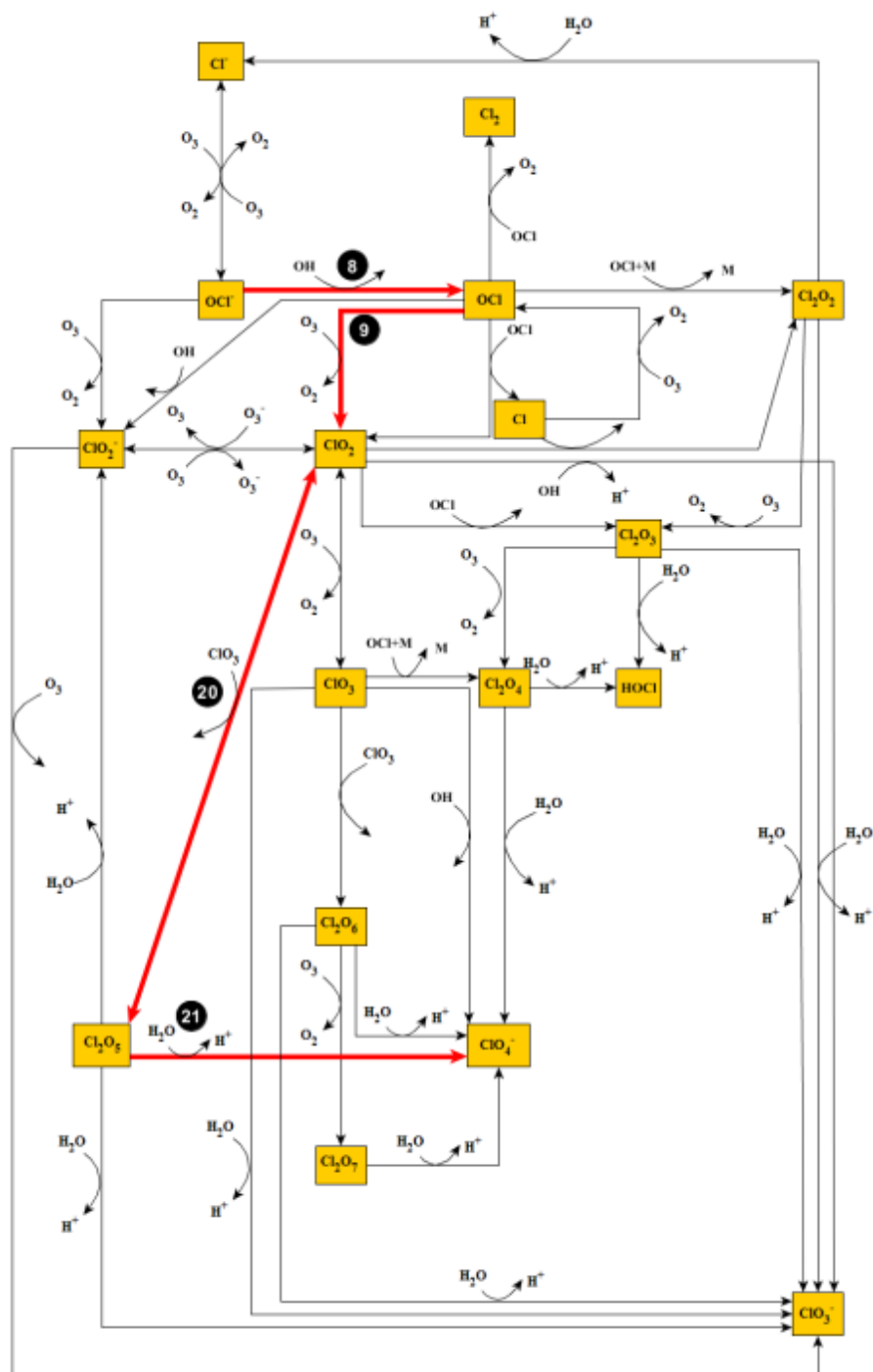


Figure A.42 Mechanism 16:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick red lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

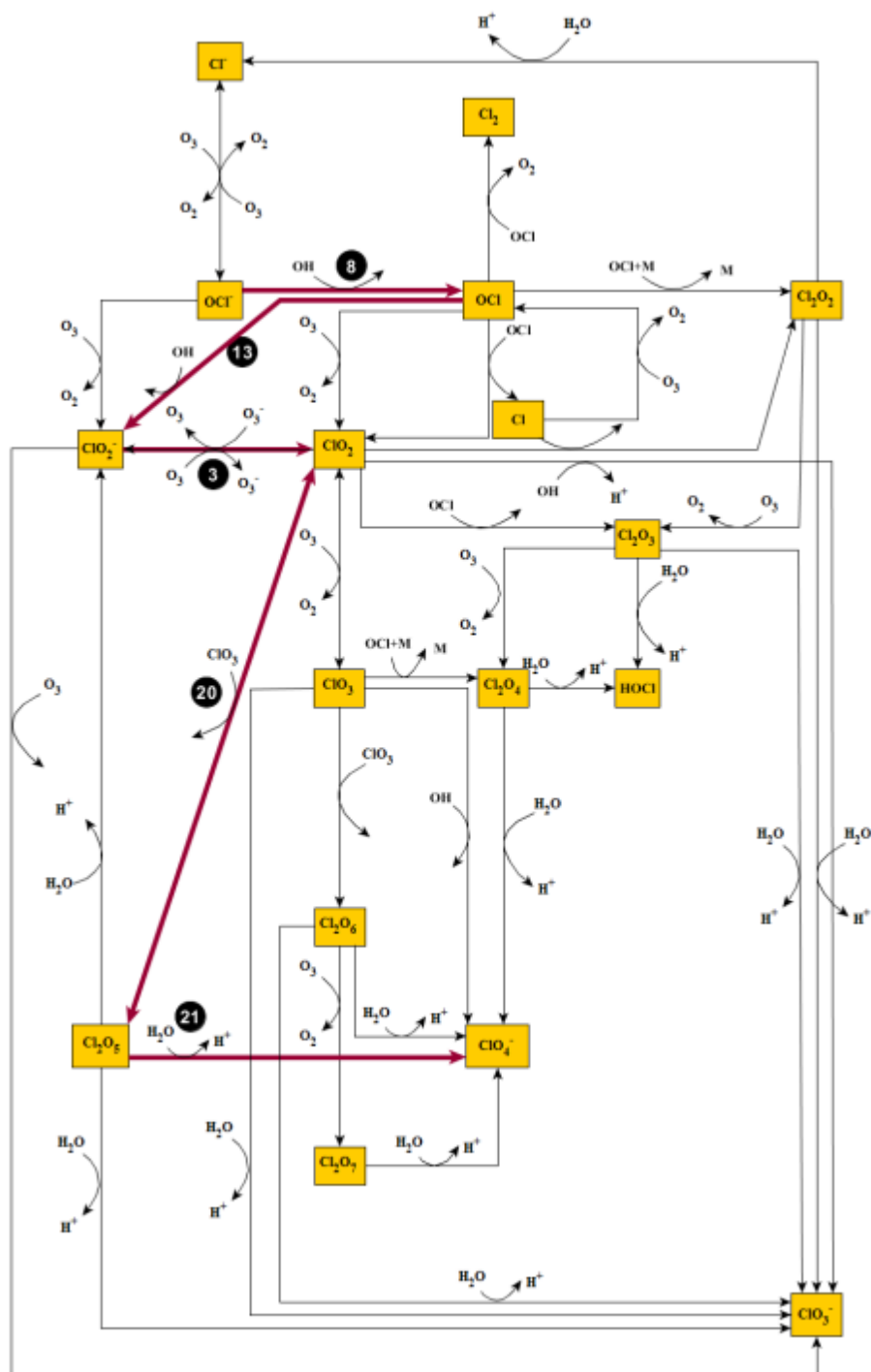


Figure A.43 Mechanism 17:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .



### A.2.4 Pathways Involving Speculated Reactions 16 and 19

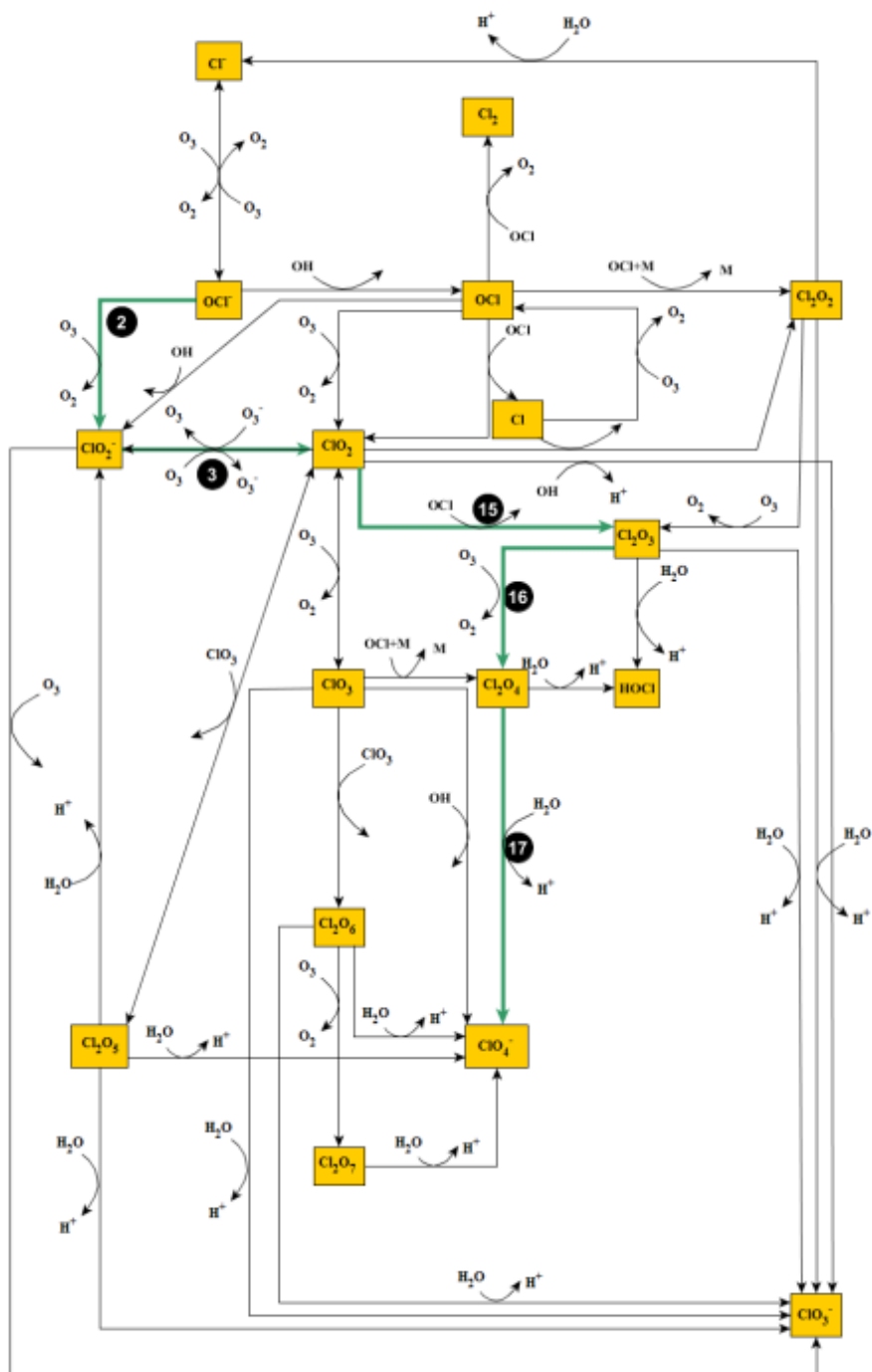


Figure A.44 Mechanism 18:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick blue green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

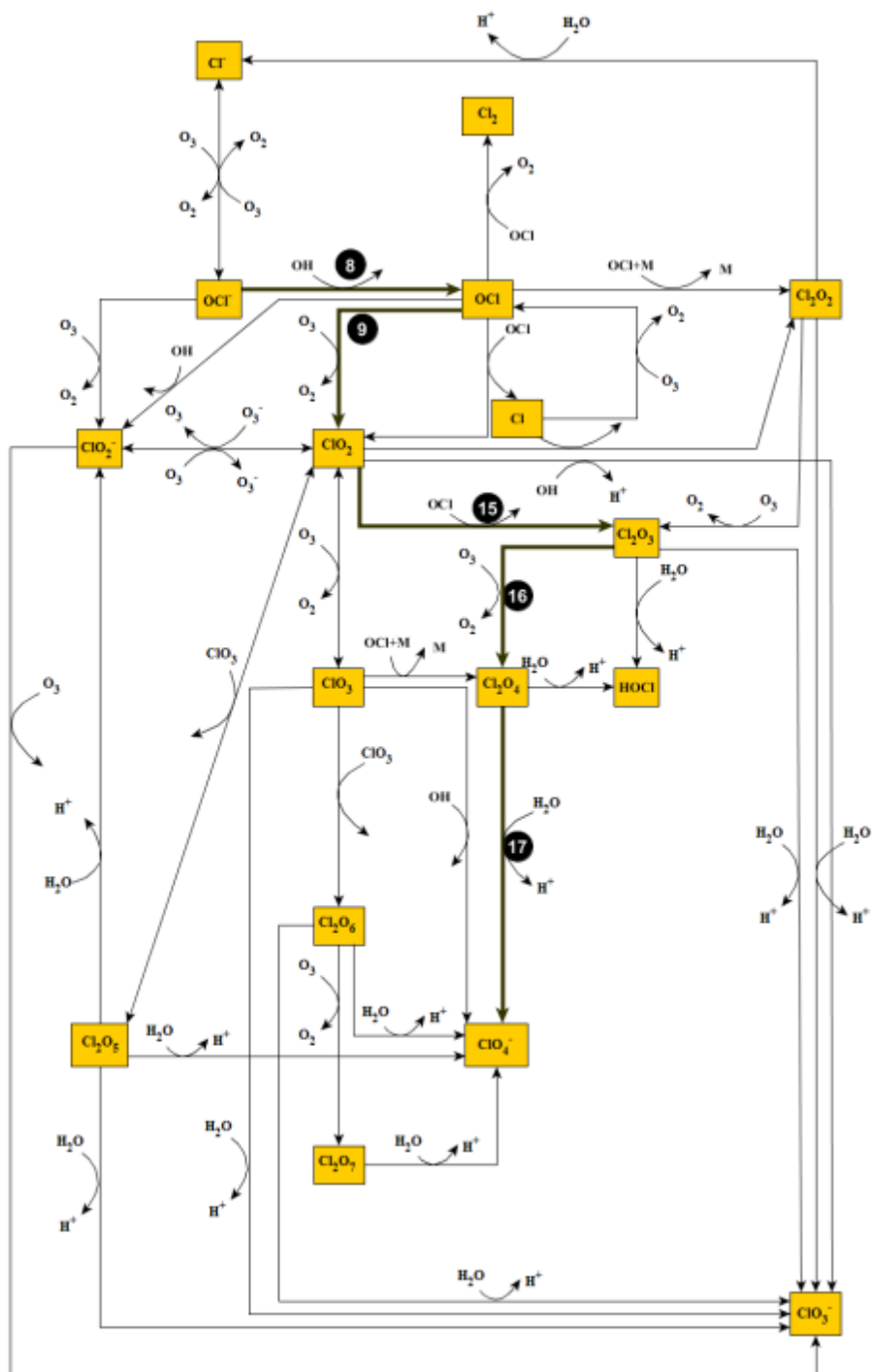


Figure A.45 Mechanism 19:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick gray lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

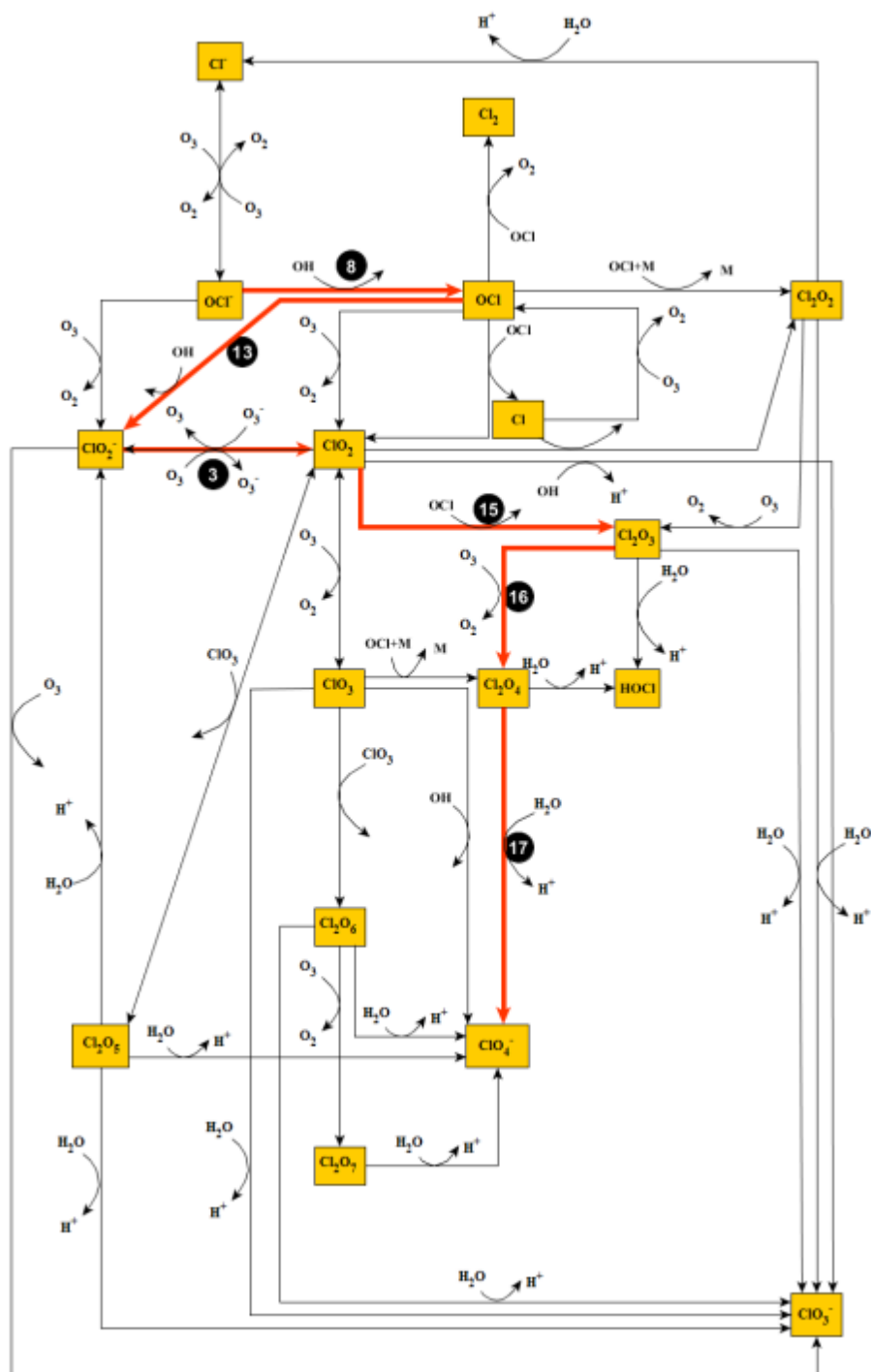


**Figure A.46 Mechanism 20:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq).** Note the thick red lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

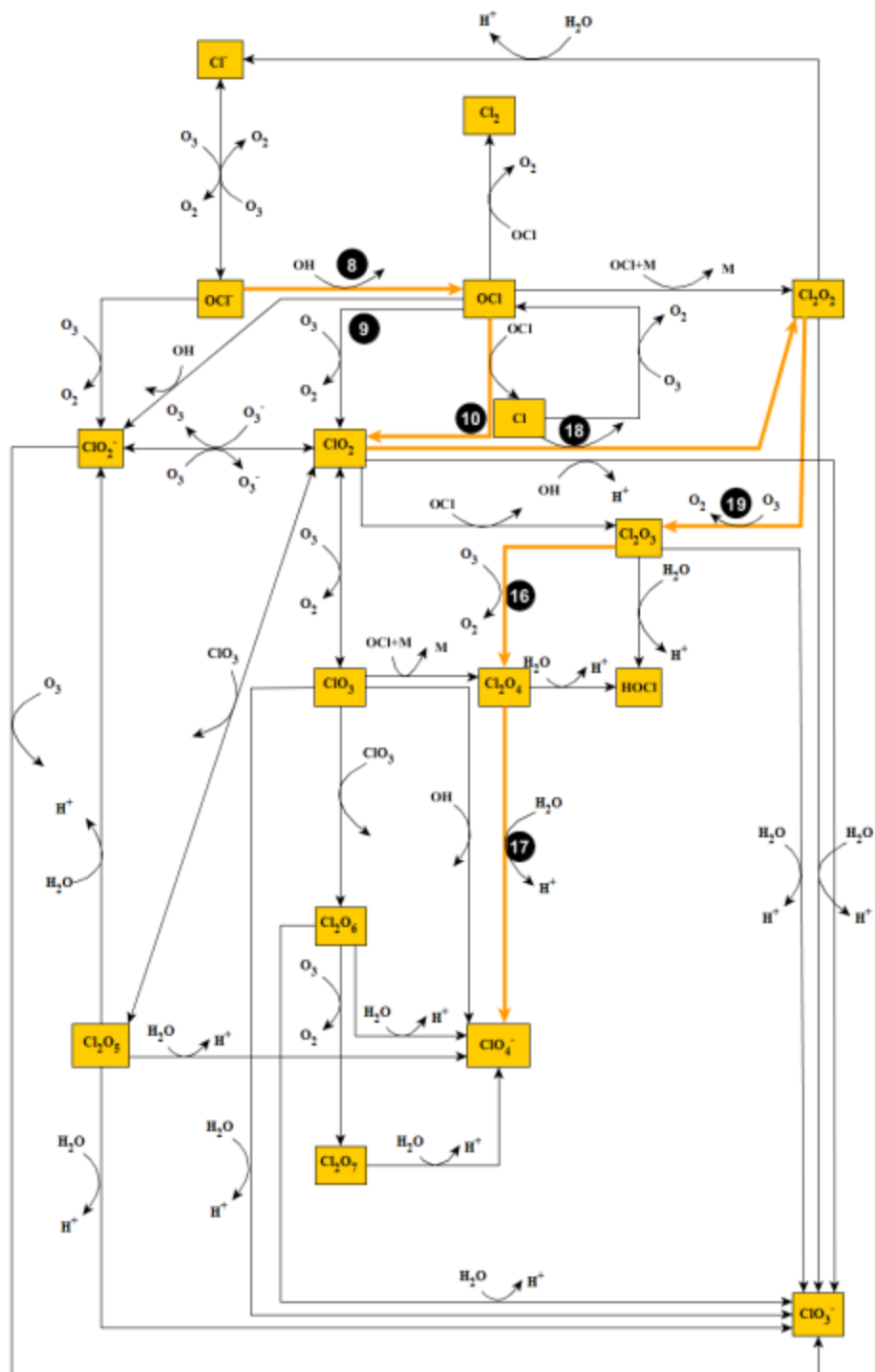


Figure A.47 Mechanism 21:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick orange lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

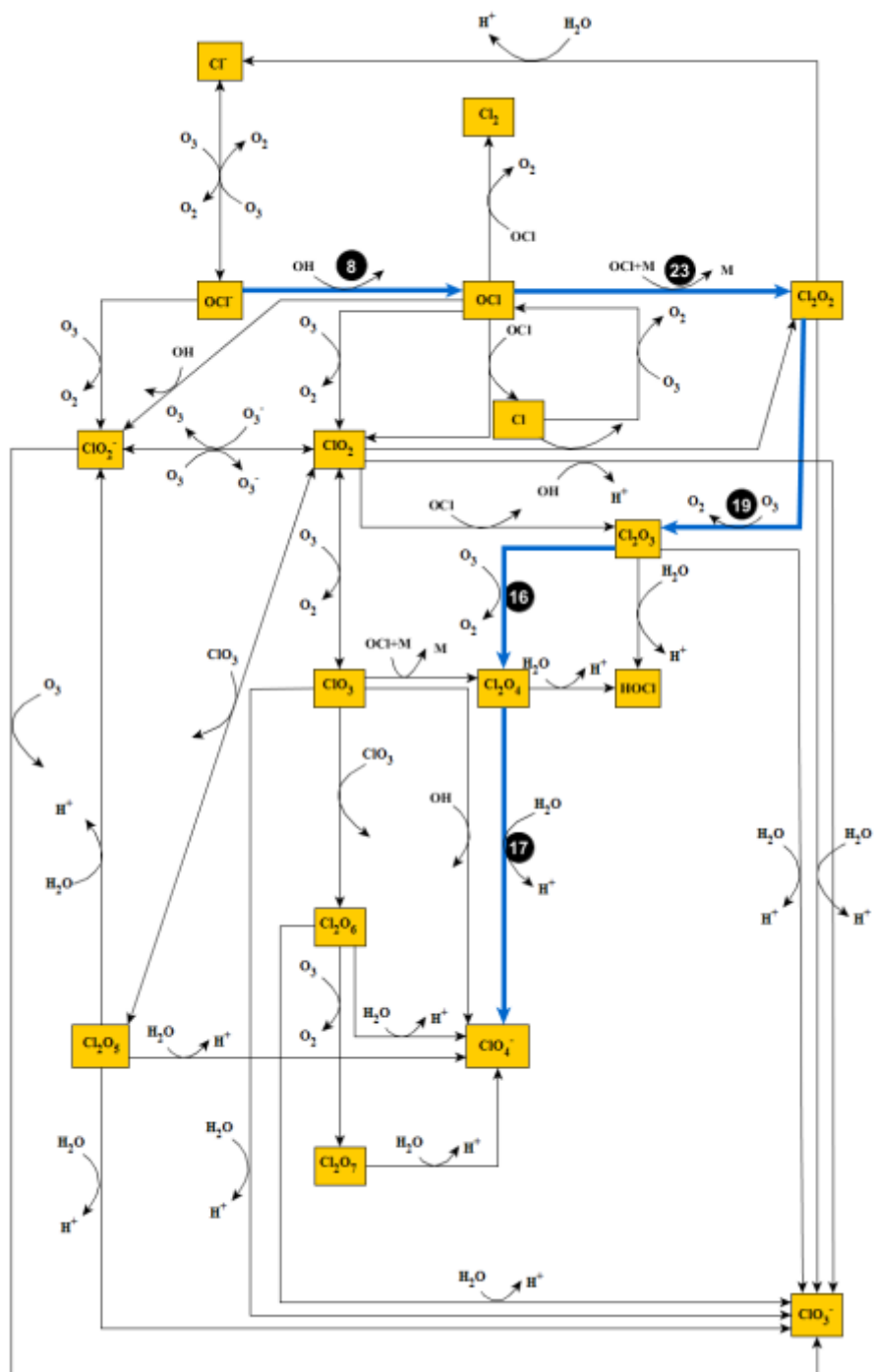


Figure A.48 Mechanism 22:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

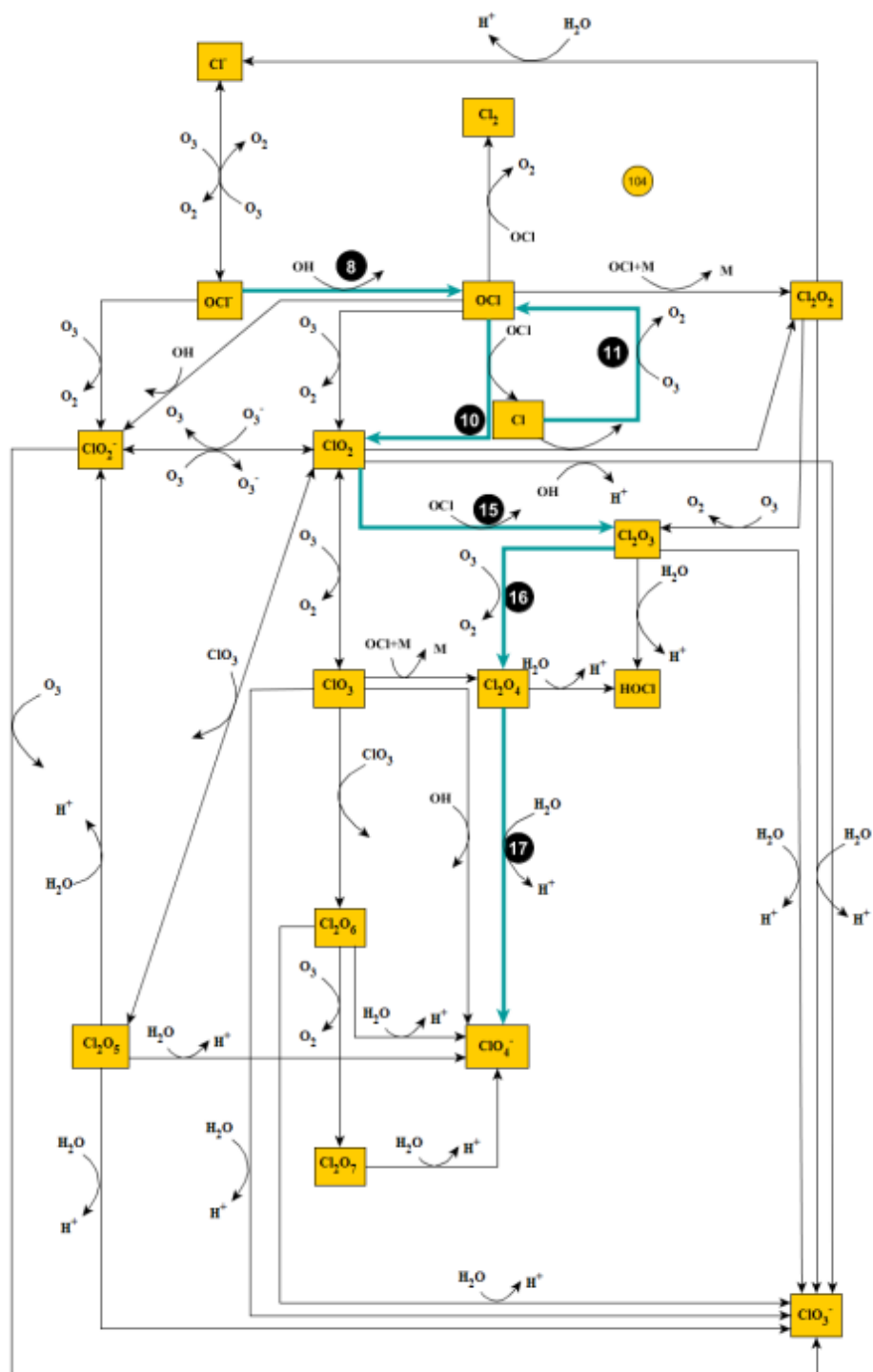


Figure A.49 Mechanism 23:  $\text{O}_3$  oxidation of  $\text{OCl}^-$  (aq). Note the thick aqua lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of one  $\text{ClO}_4^-$  molecule containing two O's from  $\text{O}_3$ .

### A.3 Proposed Pathways for O<sub>3</sub> Oxidation of Aqueous ClO<sub>2</sub><sup>-</sup>

#### A.3.1 Pathways Producing a ClO<sub>4</sub><sup>-</sup> with only two O from O<sub>3</sub>

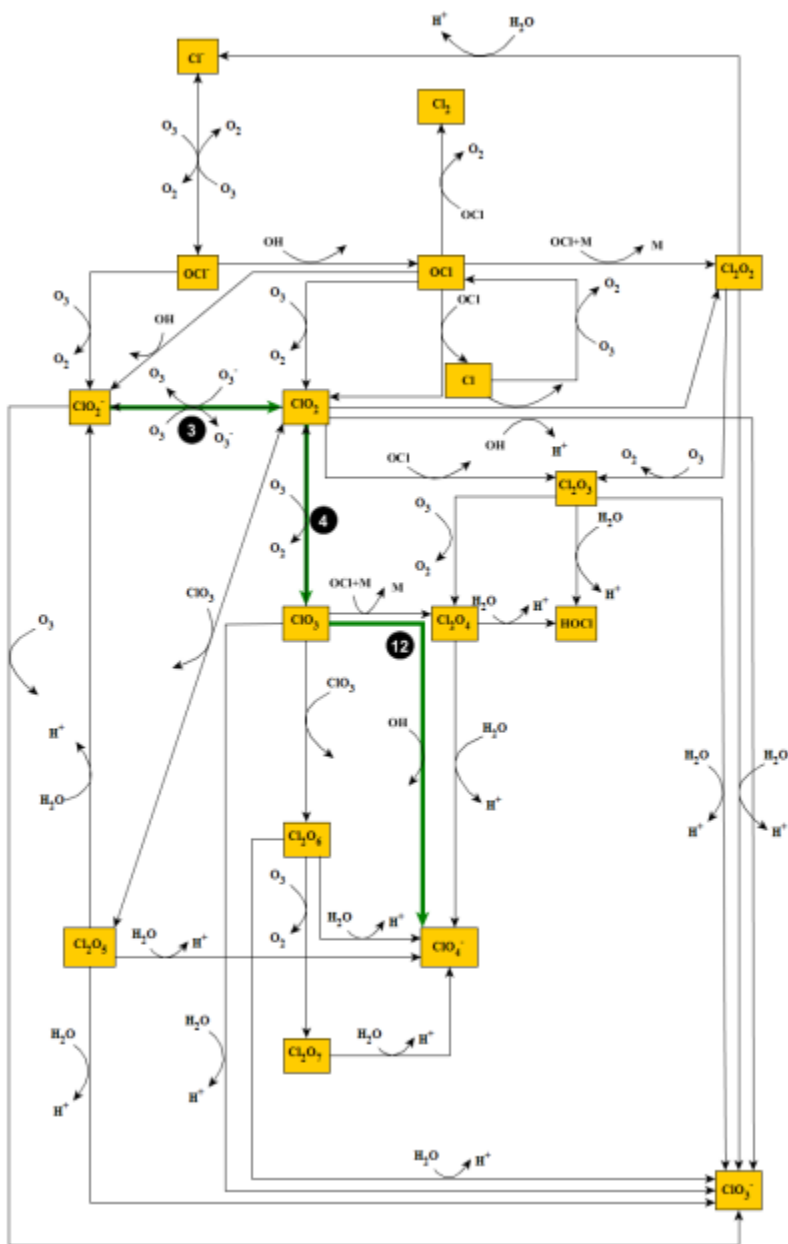


Figure A.50 Mechanism 1: O<sub>3</sub> oxidation of ClO<sub>2</sub><sup>-</sup> (aq). Note the thick orange lines indicate the pathway leading to the formation of the ClO<sub>4</sub><sup>-</sup> and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO<sub>4</sub><sup>-</sup> molecule containing two O from O<sub>3</sub>.

### A.3.2 Pathways Producing two $\text{ClO}_4^-$ molecules, one with only one O from $\text{O}_3$ and the other with two

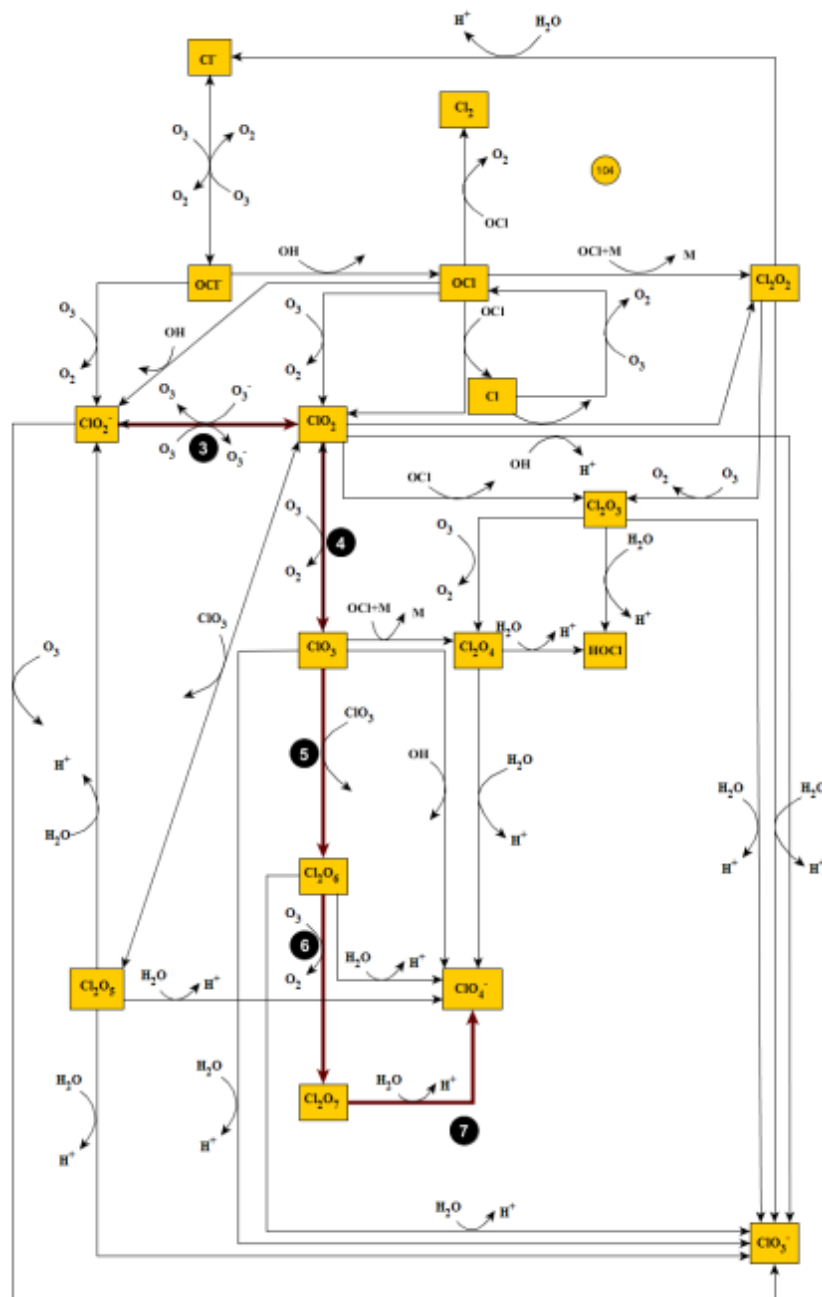


Figure A.51 Mechanism 2:  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  (aq). Note the thick maroon lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$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



### A.3.3 Pathways Producing $\text{ClO}_4^-$ with only one O from $\text{O}_3$ .

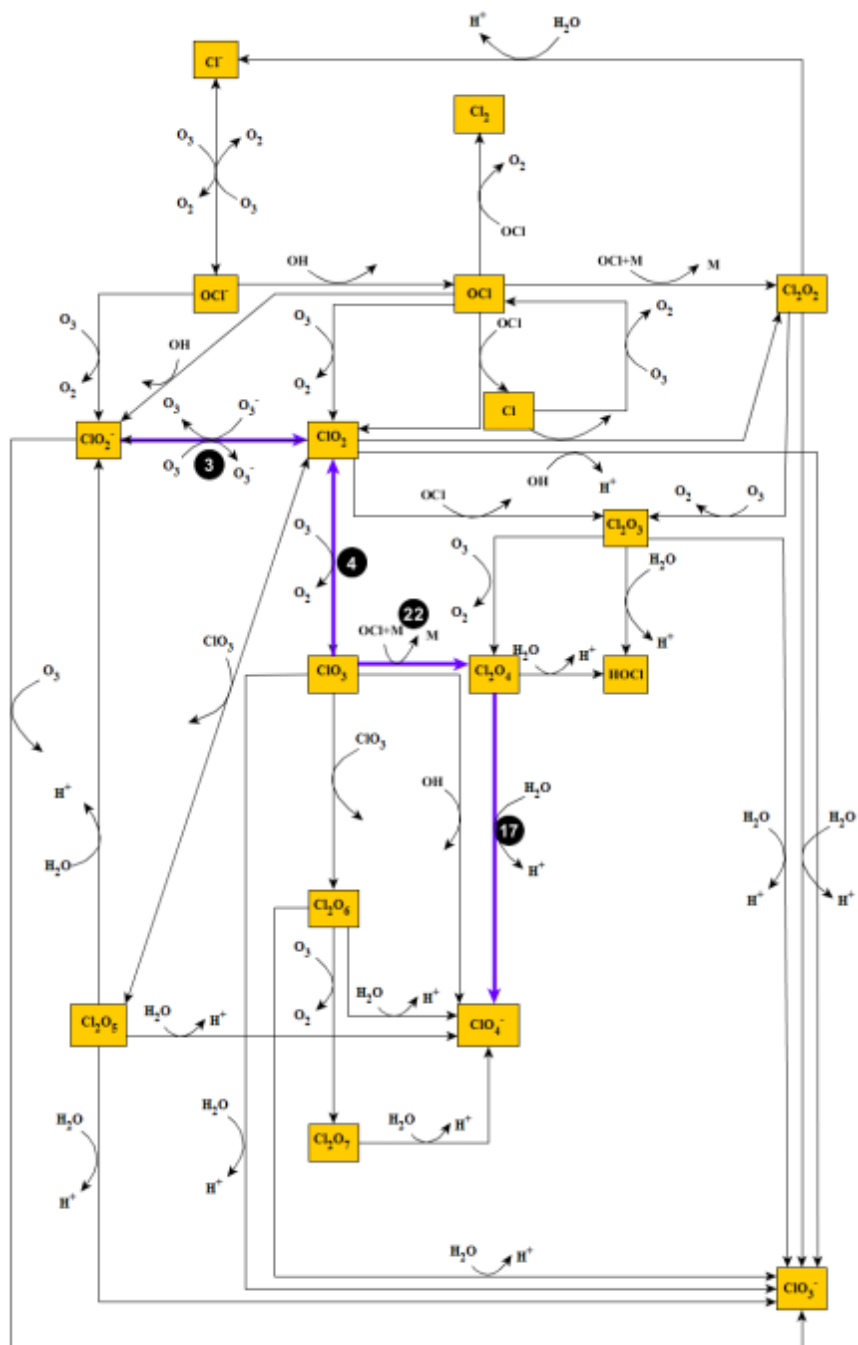


Figure A.52 Mechanism 3:  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  (aq). Note the thick purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing one O from  $\text{O}_3$ .

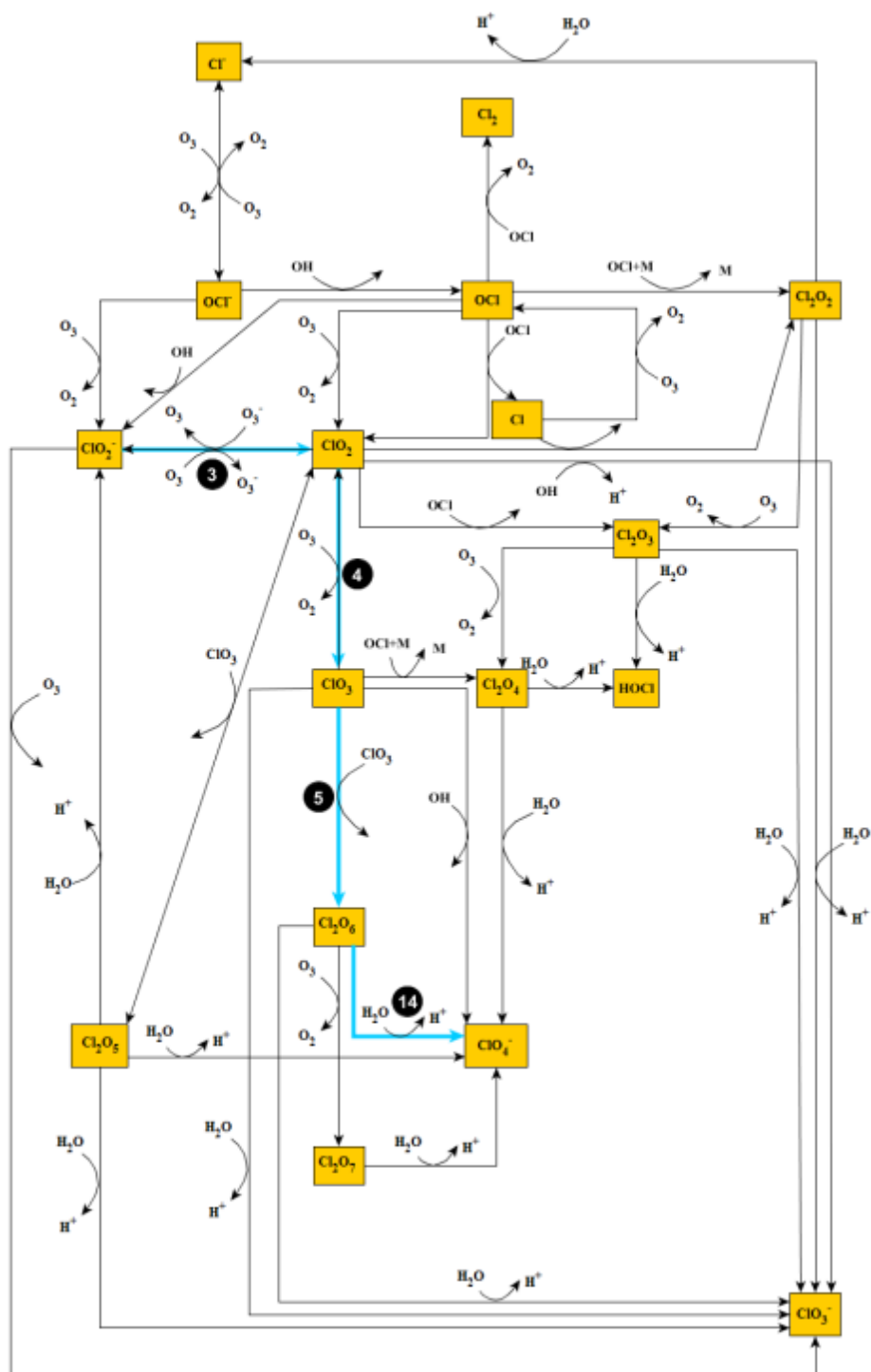


Figure A.53 Mechanism 4:  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  (aq). Note the thick light blue lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing one O from  $\text{O}_3$  and one  $\text{ClO}_3^-$  molecule containing one O from  $\text{O}_3$ .

### A.3.4 Pathways Involving the $\text{Cl}_2\text{O}_5$ species

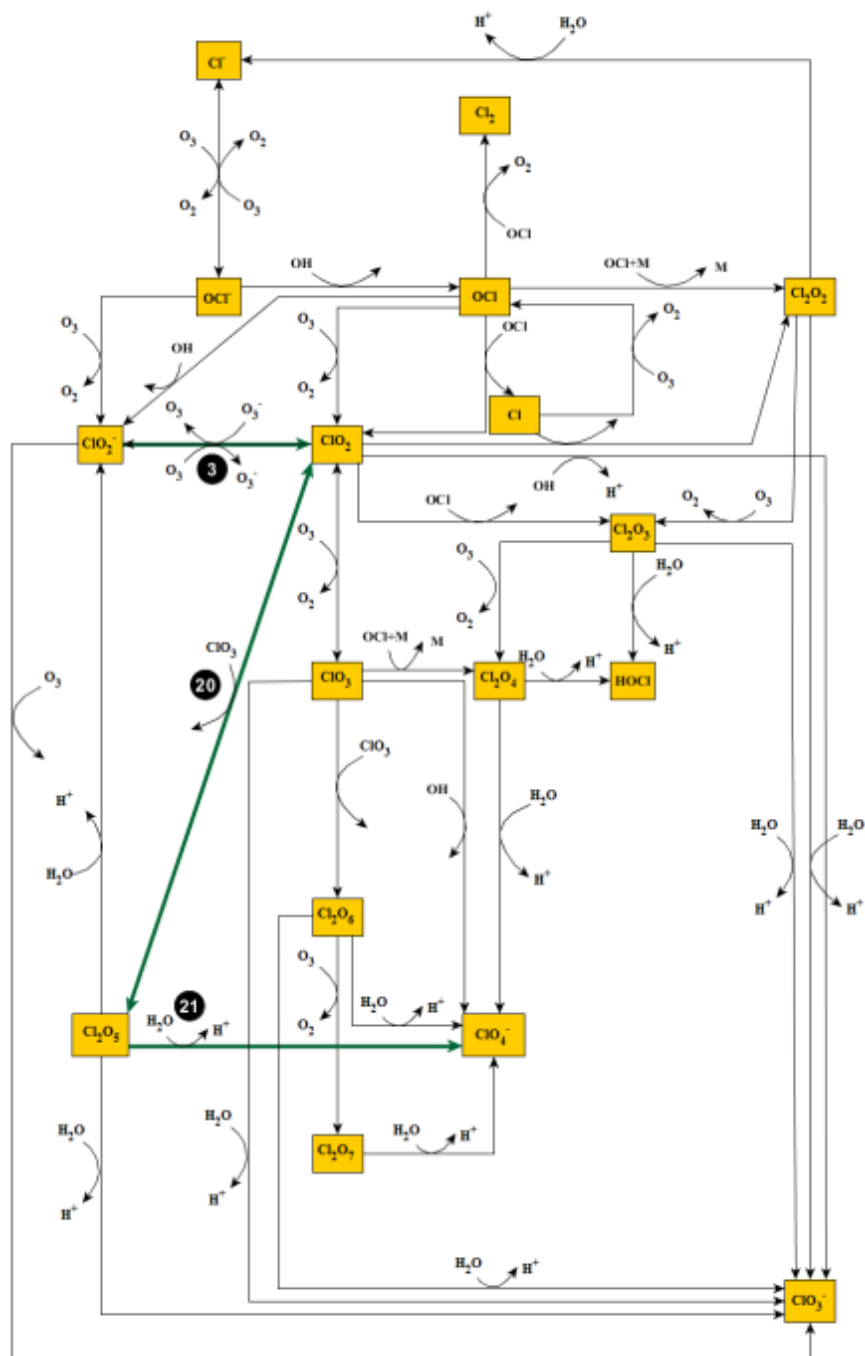


Figure A.54 Mechanism 5:  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  (aq). Note the thick green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing one O from  $\text{O}_3$ .

### A.3.5 Pathways Involving Reactions 16 and 19

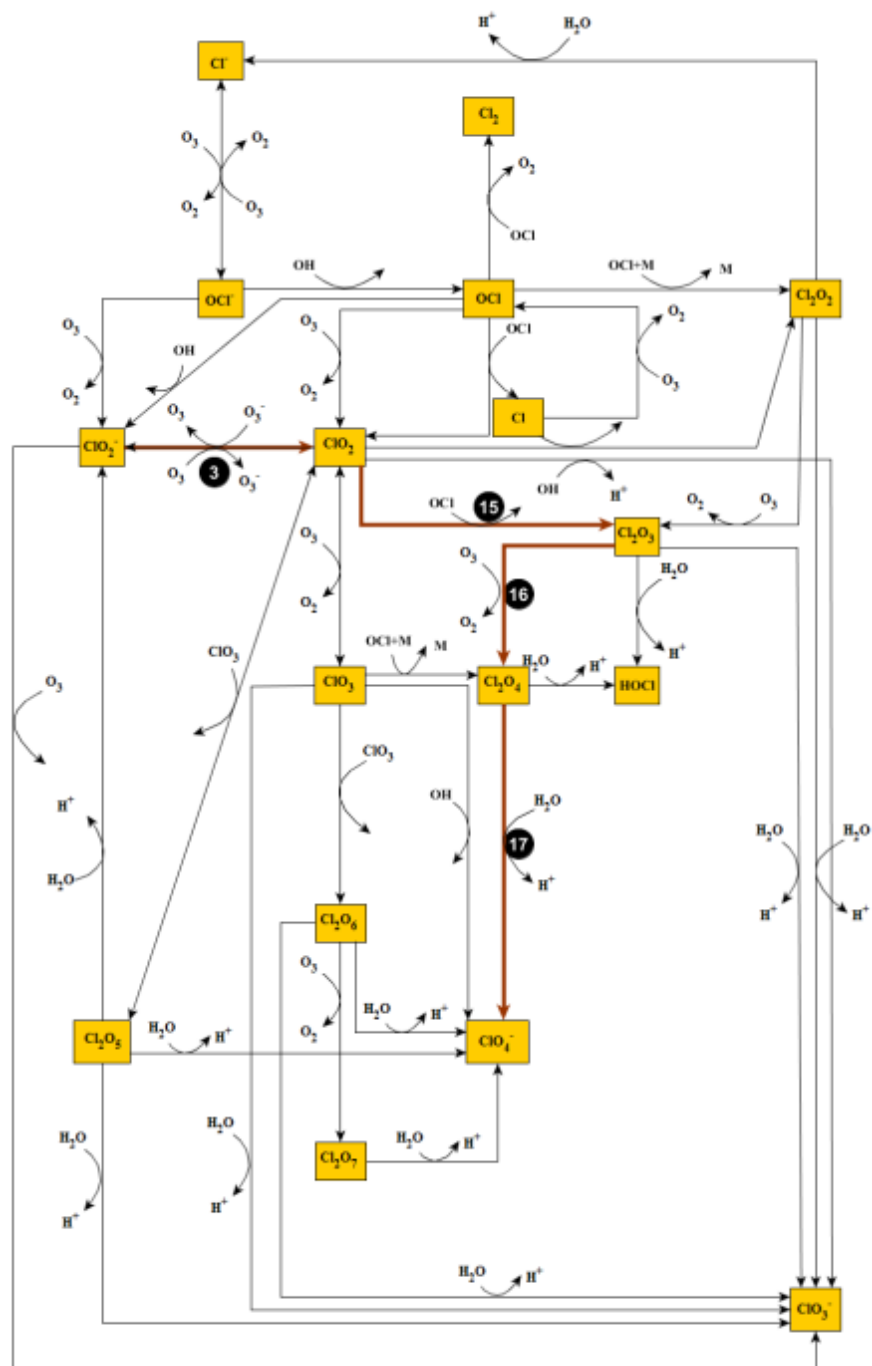


**Figure A.55 Mechanism 6:  $\text{O}_3$  oxidation of  $\text{ClO}_2^-$  (aq).** Note the thick orange lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing one O from  $\text{O}_3$ .

## A.4 Proposed Pathways for O<sub>3</sub> Oxidation of Aqueous ClO<sub>2</sub>

### A.4.1 Pathways Producing a ClO<sub>4</sub><sup>-</sup> with only two O from O<sub>3</sub>

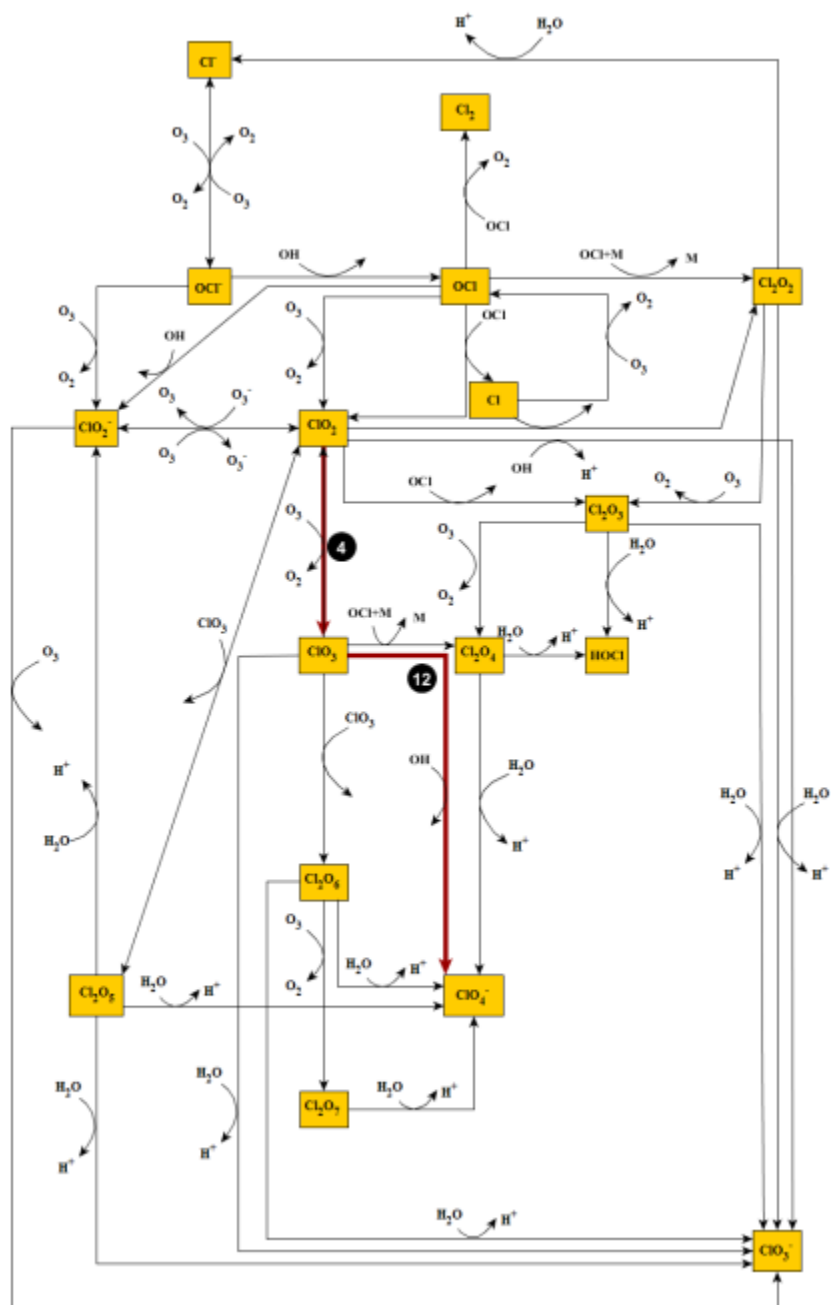


Figure A.56 Mechanism 1: O<sub>3</sub> oxidation of ClO<sub>2</sub>(aq). Note the thick maroon lines indicate the pathway leading to the formation of the ClO<sub>4</sub><sup>-</sup> and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one ClO<sub>4</sub><sup>-</sup> molecule containing two O from O<sub>3</sub>.

#### A.4.2 Pathways Producing a $\text{ClO}_4^-$ with one or two O from $\text{O}_3$

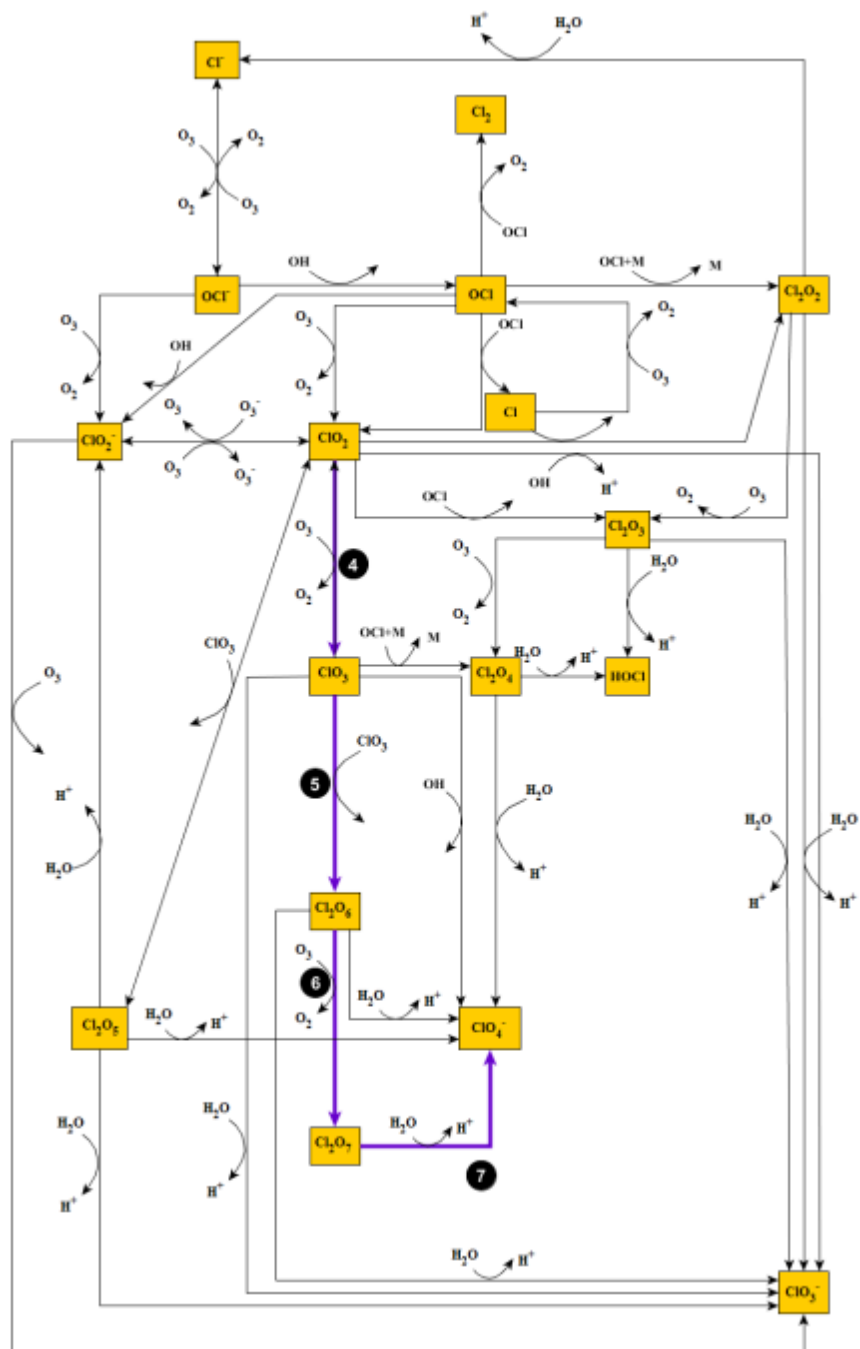


Figure A.57 Mechanism 2:  $\text{O}_3$  oxidation of  $\text{ClO}_2$  (aq). Note the thick purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation of two  $\text{ClO}_4^-$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

#### A.4.3 Pathways Producing a $\text{ClO}_4^-$ with only one O from $\text{O}_3$ .

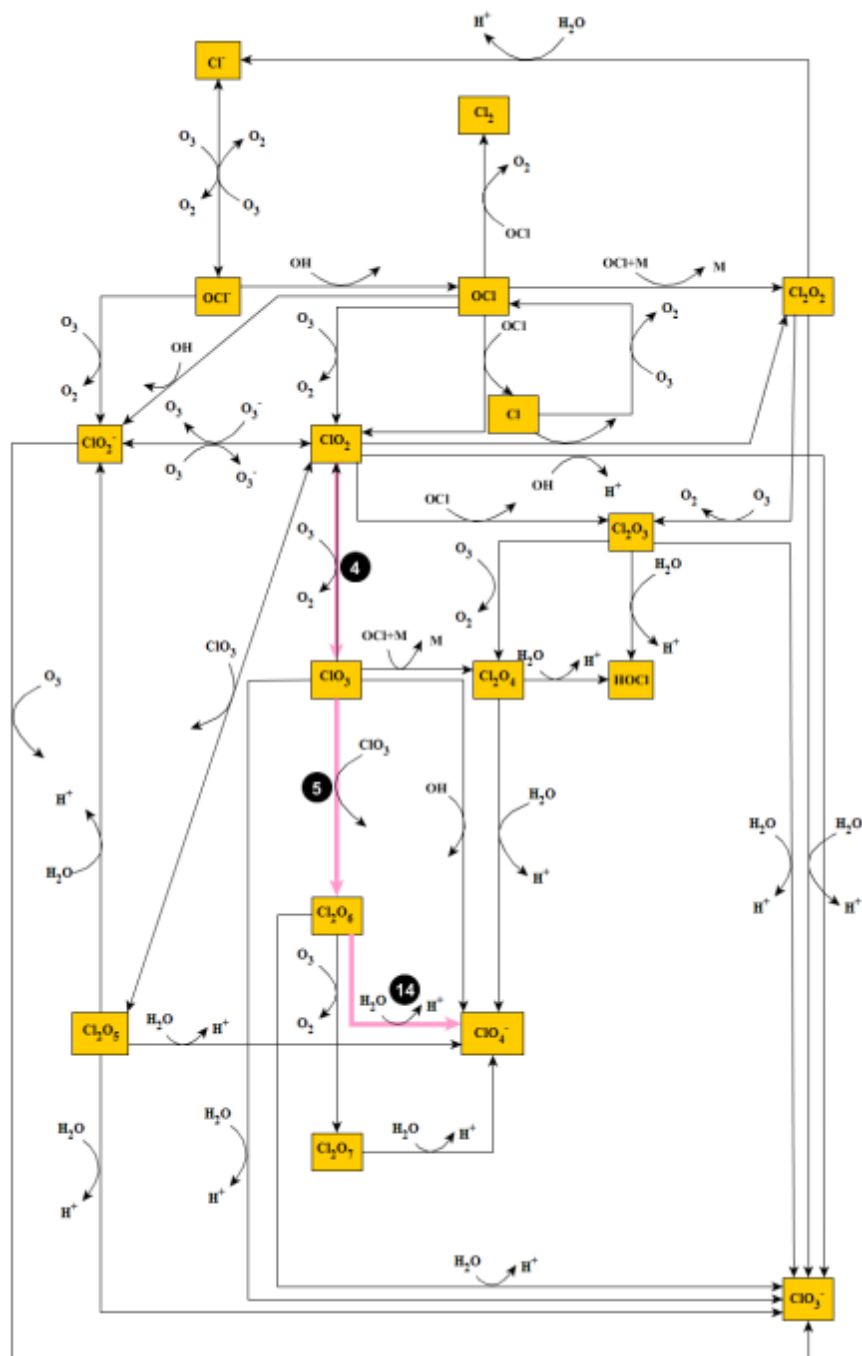


**Figure A.58 Mechanism 3:  $\text{O}_3$  oxidation of  $\text{ClO}_2(\text{aq})$ .** Note the thick pink lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing one O from  $\text{O}_3$  and one  $\text{ClO}_3^-$  molecule containing one O from  $\text{O}_3$ .

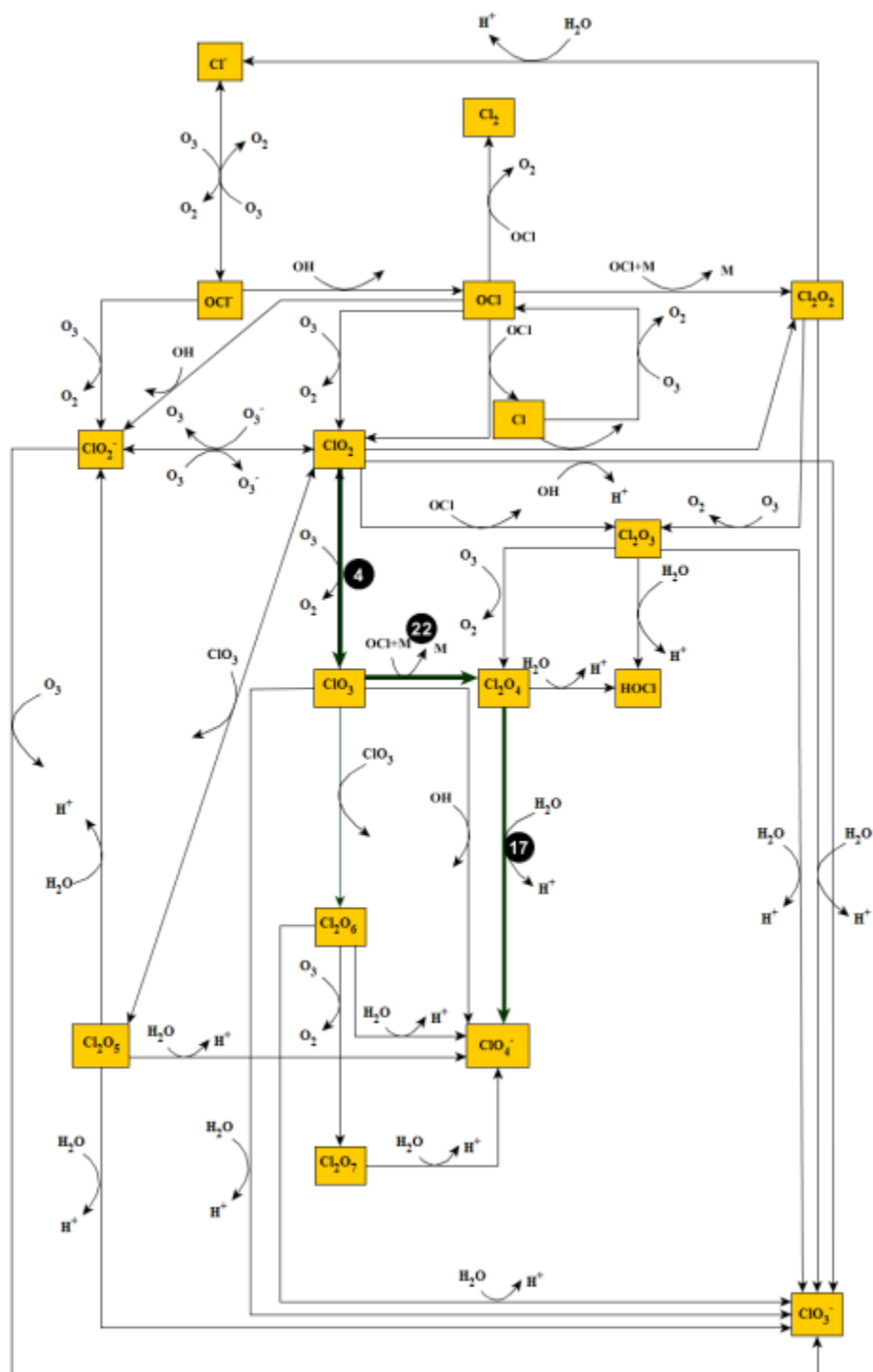


Figure A.59 Mechanism 4:  $\text{O}_3$  oxidation of  $\text{ClO}_2(\text{aq})$ . Note the thick green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing one O from  $\text{O}_3$ .



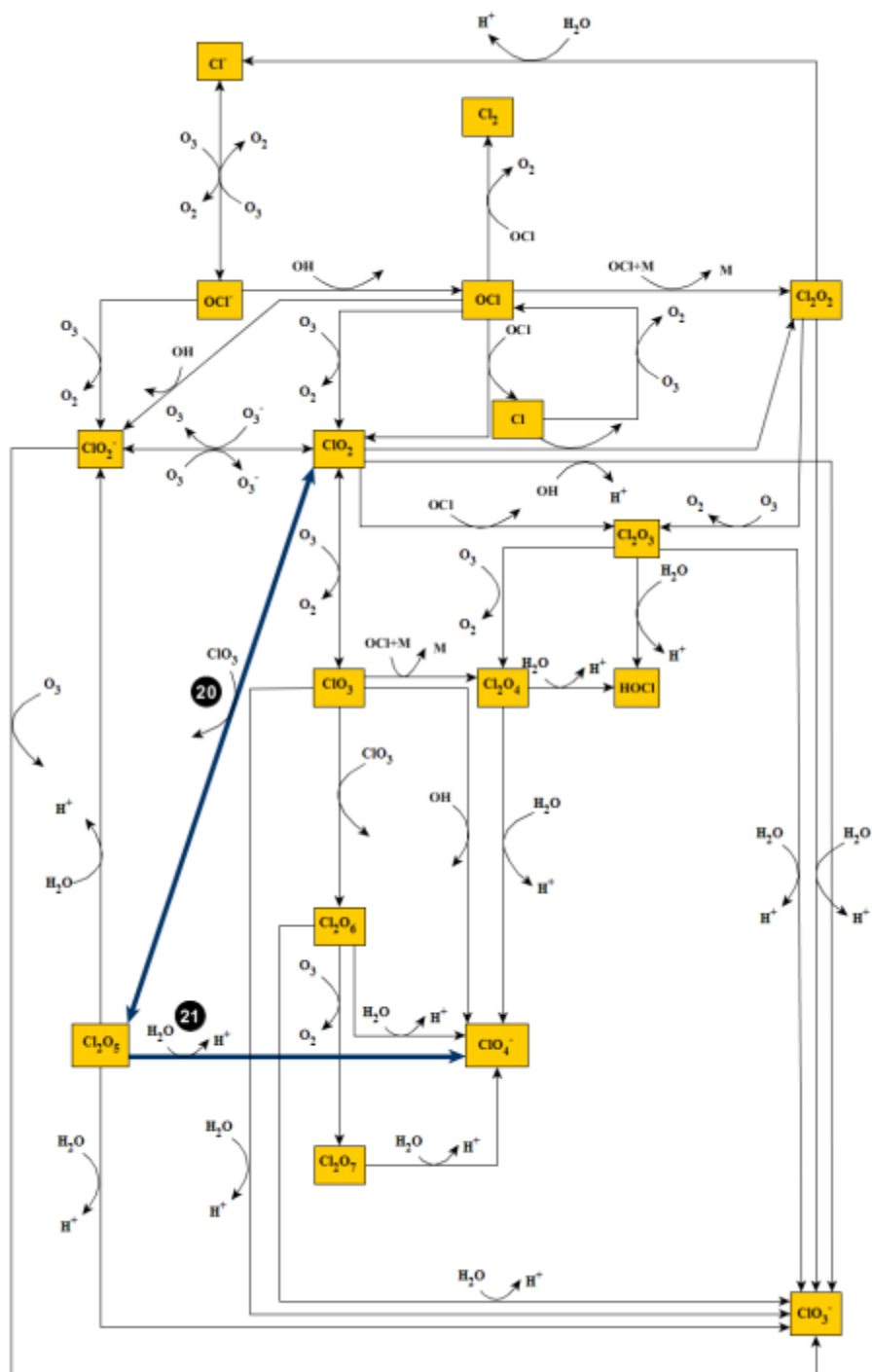


**Figure A.60 Mechanism 5:  $\text{O}_3$  oxidation of  $\text{ClO}_2$  (aq).** Note the thick purple lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing one O from  $\text{O}_3$ .

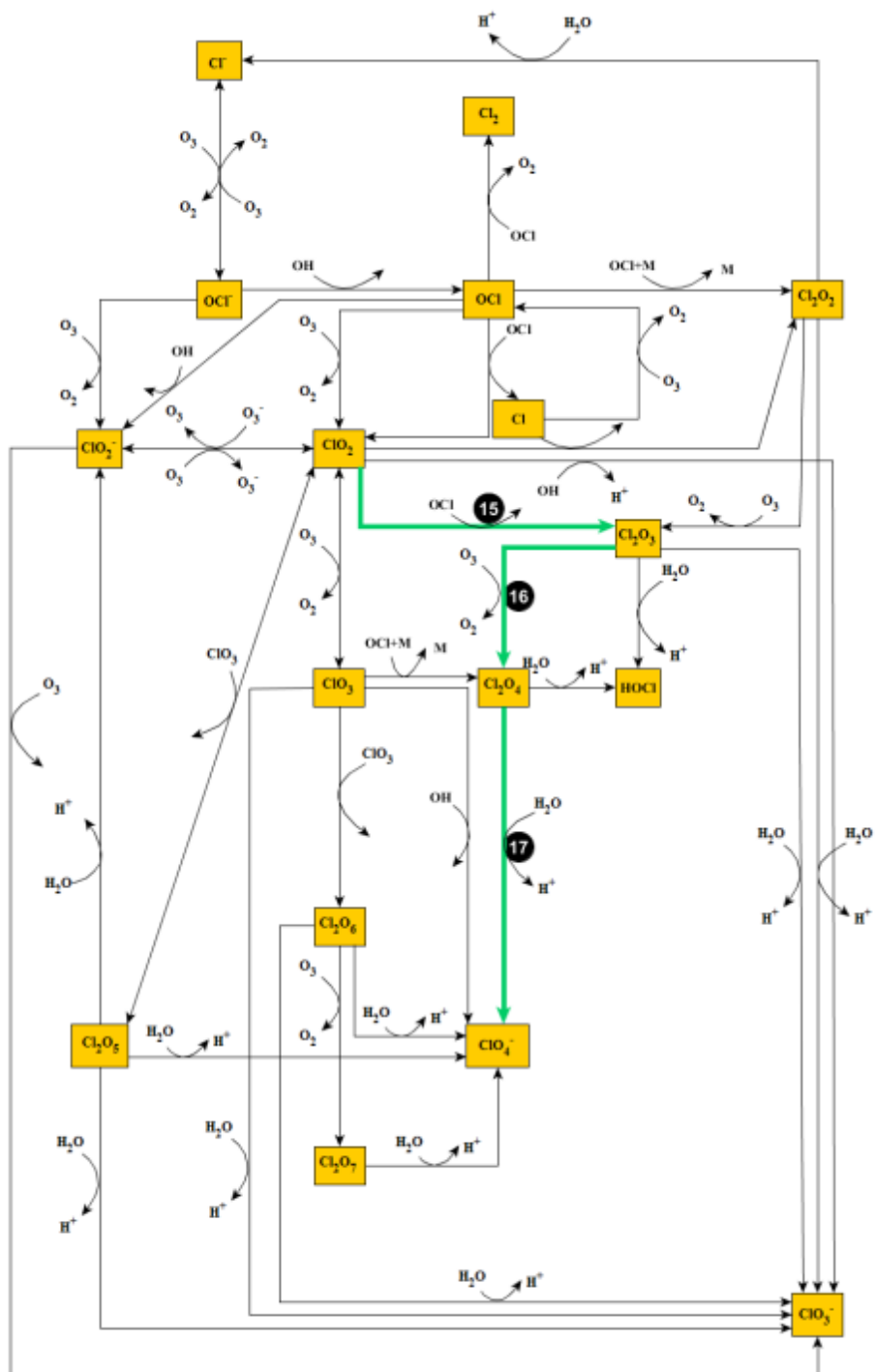


Figure A.61 Mechanism 6:  $\text{O}_3$  oxidation of  $\text{ClO}_2$  (aq). Note the thick green lines indicate the pathway leading to the formation of the  $\text{ClO}_4^-$  and black solid circles represent reaction number from Table 3.4. This pathway leads to the formation one  $\text{ClO}_4^-$  molecule containing one O from  $\text{O}_3$ .