

7-1-2016

Perchlorate and volatiles of the brine of Lake Vida (Antarctica): Implication for the in situ analysis of Mars sediments

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Recommended Citation

Kenig, F., Chou, L., McKay, C., Jackson, W., Doran, P., Murray, A., & Fritsen, C. (2016). Perchlorate and volatiles of the brine of Lake Vida (Antarctica): Implication for the in situ analysis of Mars sediments. *Journal of Geophysical Research: Planets*, 121 (7), 1190-1203. <https://doi.org/10.1002/2015JE004964>

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RESEARCH ARTICLE

10.1002/2015JE004964

Key Points:

- The brine of Lake Vida (Antarctica) contains oxychlorines complicating the study of volatiles
- Carbon-limited experiments do not provide data on the effects of oxychlorine breakdown
- High reduced-carbon/oxychlorine ratio is needed to detect organics on Mars via pyrolysis if these reactants cooccur

Supporting Information:

- Figures S1 and S2

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Citation:

Kenig, F., L. Chou, C. P. McKay, W. A. Jackson, P. T. Doran, A. E. Murray, and C. H. Fritsen (2016), Perchlorate and volatiles of the brine of Lake Vida (Antarctica): Implication for the in situ analysis of Mars sediments, *J. Geophys. Res. Planets*, 121, 1190–1203, doi:10.1002/2015JE004964.

Received 22 OCT 2015

Accepted 15 JUN 2016

Accepted article online 17 JUN 2016

Published online 5 JUL 2016

Perchlorate and volatiles of the brine of Lake Vida (Antarctica): Implication for the in situ analysis of Mars sediments

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Abstract The cold (-13.4°C), cryoencapsulated, anoxic, interstitial brine of the >27 m thick ice of Lake Vida (Victoria Valley, Antarctica) contains $49\ \mu\text{g}\cdot\text{L}^{-1}$ of perchlorate and $11\ \mu\text{g}\cdot\text{L}^{-1}$ of chlorate. Lake Vida brine (LVBr) may provide an analog for potential oxychlorine-rich subsurface brine on Mars. LVBr volatiles were analyzed by solid-phase microextraction (SPME) gas chromatography–mass spectrometry (GC-MS) with two different SPME fibers. With the exception of volatile organic sulfur compounds, most other volatiles observed were artifacts produced in the GC injector when the thermal decomposition products of oxychlorines reacted with reduced carbon derived from LVBr and the SPME fiber phases. Analysis of MilliQ water with perchlorate ($40\ \mu\text{g}\cdot\text{L}^{-1}$) showed low level of organic artifacts, reflecting carbon limitation. In order to observe sample-derived organic compounds, both in analog samples and on Mars, the molar abundance of reduced carbon in a sample must exceed those of O_2 and Cl_2 produced during decomposition of oxychlorines. This suggests that the abundance of compounds observed by the Sample Analysis at Mars (SAM) instruments in Sheepbed samples (CB-3, CB5, and CB6) may be controlled by an increase in the reduced-carbon/oxychlorine ratio of these samples. To increase chances of in situ detection of Martian organics during pyrolysis-GC-MS, we propose that the derivatization agents stored on SAM may be used as an external source of reduced carbon, increasing artificially the reduced-carbon to perchlorate ratio during pyrolysis, allowing the expression of more abundant and perhaps more diverse Martian organic matter.

1. Introduction

Since the recent discovery of oxychlorines in surface sediments of Mars [Hecht *et al.*, 2009], the methylchloride (MeCl) and dichloromethane (DCM) observed in a Martian soil sample during the Viking missions upon thermal desorption and pyrolysis gas chromatography–mass spectrometry (GC-MS) [Biemann *et al.*, 1977] were reinterpreted to be the products of oxychlorine decomposition and reaction with Martian carbon during analysis [Navarro-Gonzalez *et al.*, 2010]. The apparent ubiquity of oxychlorines on Mars surface [Archer *et al.*, 2015; Stern *et al.*, 2015; Ming *et al.*, 2014; Hecht *et al.*, 2009] is a challenge to our ability to detect and quantify organic compounds with the instruments dedicated to organic matter analysis on the Curiosity Rover. These instruments use thermal desorption and pyrolysis and, at temperatures exceeding 240°C , oxychlorines decompose, oxidizing the available reduced carbon and generating CO_2 ; HCl; and chlorinated organic species such as MeCl, DCM, and trichloromethane (TCM) [Glavin *et al.*, 2013; Leshin *et al.*, 2013]. Both chlorinated and nonchlorinated compounds detected at Rocknest and Yellowknife bay by the Sample Analysis at Mars (SAM) instruments on the Curiosity Rover were considered to be directly derived from the instrument itself or to be the products of oxychlorine degradation and reaction with organic components of the pyrolysis-trap-gas chromatography system used [Glavin *et al.*, 2013; Leshin *et al.*, 2013]. Additionally, the by-product of oxychlorine degradation did react with N-methyl-N-(tert-butylidimethylsilyl)-trifluoroacetamide (MTBSTFA), a leaking derivatizing agent [Leshin *et al.*, 2013]. Recently, Freissinet *et al.* [2015] showed that the chlorinated compounds observed in the Sheepbed Mudstone at Gale Crater, chlorobenzene and dichloroalkanes, are derived from the reaction of Martian oxychlorines with Martian organic carbon. This hypothesis was supported by the greater abundance of these chlorinated compounds relative to those observed in previous sediment samples analyzed, their absence from blanks, and the direct detection of chlorobenzene by evolved gas analysis (EGA).

For now, the abundance and diversity of all the compounds observed by SAM's GC-MS are small. *Benner et al.* [2000] predicted that water cleavage by ultraviolet radiation would produce OH[•] and H[•] radicals able capable of oxidizing the organic matter brought to Mars via meteor, resulting in the formation of organic acids with recalcitrant end-products such as mellitic and phthalic acids. *Benner et al.* [2000] also suggest that surface diagenesis of organic matter derived from potential abiotic processes or from hypothetical life would follow the same level of oxidation, reducing considerably the potential diversity and abundance of organic compounds on the surface of Mars. More recently, *Stalport et al.* [2010] showed that compounds such as mellitic and phthalic acids are not resistant to solar UV exposures. In contrast, recent experiments by *Poch et al.* [2014] showed that some compounds such as chrysene or adenine are more resistant to the Martian radiation regime.

The paucity of Martian organic compounds currently detected on Mars may also suggest that the reduced-carbon/oxychlorine molar ratio in Martian samples is generally small enough to prevent Martian organic compounds from being observed by pyrolysis-trap-GC-MS. In the presence of an excess of oxychlorines relative to reduced carbon during pyrolysis, organic compounds could completely oxidize to CO₂ and C₁ chlorinated compounds.

Oxychlorines on Earth do not accumulate significantly away from desert regions because they are leached by precipitation water and are reduced biotically and abiotically [*Coates and Achenbach*, 2004]. The abundance of oxychlorines in surface sediments of Mars (from 0.1 to ~2 wt % ClO₄⁻ [*Stern et al.*, 2015; see also *Ming et al.*, 2014]) suggests that neither microbial (if any) and abiotic reduction nor leaching of these compounds is taking place at a significant rate and thus suggests that the rate of oxychlorine formation exceeds that of their destruction [*Catling et al.*, 2010]. On Mars, if the waters in which lacustrine sediments accumulated contained oxychlorines, interstitial waters of lacustrine sediments would also contain oxychlorines, and upon water loss, the oxychlorines would have now precipitated at the surface of the sediments and within sediment pore space. Thus, it is likely that oxychlorines will be pervasive in all Mars surface lake sediments. Similarly, subglacial brines or interstitial brines on Mars would also contain oxychlorines, as these brines are the evaporation products of open water that would have contained oxychlorines. In situ detection of perchlorates in the soils of polar Mars by the Phoenix Mars Lander [*Hecht et al.*, 2009] led *Fisher et al.* [2010] to hypothesize that perchlorates may contribute to melting the base of the ice caps and generate brines. Further, *Fisher et al.* [2010] suggest that these oxychlorine-rich brines could collect, mobilize, and form networks of pools or interstitial brines within the sediments under the ice cap.

The McMurdo Dry Valleys (MDVs; East Antarctica) constitute the largest area (~5000 km²) of Antarctica not covered by glacier ice. The dry valleys are one of the driest and coldest environments on Earth with mean annual precipitation of less than 50 mm [*Fountain et al.*, 2010], sublimation exceeding precipitation, and mean annual temperatures ranging from -30 to -14.8°C on the valley floor (the low end of this range was recorded at Lake Vida [*Doran et al.*, 2002]). As a result, the dry valleys have been often considered to be an Earth analog to Mars past and present environments [e.g., *Andersen et al.*, 1994; *Bishop et al.*, 2013; *Doran et al.*, 1998].

Recently, aquifers were detected within the permafrost of some of the McMurdo Dry Valleys, Taylor Valley [*Mikucki et al.*, 2015], and Victoria Valley [*Dugan et al.*, 2015a]. These subsurface aquifers may provide a terrestrial analog for potential subsurface brines on Mars. Lake Vida is located in Victoria Valley, the northern most of the McMurdo Dry Valleys. Lake Vida appears to be entirely frozen (>27 m [*Dugan et al.*, 2015b]) and contains, 16 m below the surface, an interstitial brine at -13.4°C that supports a slow but active bacterial ecosystem [*Murray et al.*, 2012]. This interstitial brine provides a window into a potential analog of Martian brine. The anoxic brine has a high dissolved organic carbon (DOC) concentration (48.2 ± 9.7 mmol · L⁻¹ [*Murray et al.*, 2012]).

This manuscript reports on the concentrations of perchlorate and chlorate in the Lake Vida brine (LVBr) and discusses their significance in relation to the interpretation of organic matter analysis on Mars. Specifically, the artifacts created by these oxidants upon analysis of the volatiles in LVBr by direct immersion (DI)-solid-phase microextraction (SPME) GC-MS are described. The manuscript reports the results obtained upon analysis of MilliQ water containing perchlorate by DI-SPME GC-MS and discusses the serious limitations of such carbon-limited blank analysis.

The compounds observed during DI-SPME GC-MS analysis of LVBr are described in detail. The method used here, DI-SPME GC-MS of liquid samples, is different from that used by SAM (pyrolysis-trap-GC-MS of solid samples).

However, observations made and conclusions reached based on the reactions of oxychlorines and reduced carbon in our experiments can be used, in part, to better understand the data produced by SAM at Rocknest [Glavin *et al.*, 2013; Leshin *et al.*, 2013] and at Yellowknife Bay [Freissinet *et al.*, 2015; Ming *et al.*, 2014]. The manuscript debates the validity of some of the carbon-limited analog experiments made on Earth in order to test the validity of the interpretation of the data obtained by SAM. Finally, the manuscript describes an analytical strategy that would increase the chances of detecting actual Martian organic matter with SAM's pyrolysis-trap-GC-MS.

2. Samples and Methods

The brine samples analyzed were collected during the 2005–2006 and 2010–2011 expeditions to Lake Vida. The brine sampling strategy is described in Doran *et al.* [2008] and Murray *et al.* [2012]. All samples were poisoned with mercuric chloride and kept refrigerated at 4°C until analysis. ClO_4^- and ClO_3^- concentrations were measured separately by sequential ion chromatography–MS/MS (IC-MS/MS) following the method detailed in Jackson *et al.* [2010, 2012, 2015]. ClO_4^- and ClO_3^- were quantified using a Dionex LC 20 ion chromatography system consisting of GP50 pump, CD25 conductivity detector, AS40 automated sampler, and Dionex IonPac AS16 (250 × 2 mm) analytical column. The IC system was coupled with an Applied Biosystems–MDS SCIEX API 2000CX⁺ triple quadrupole mass spectrometer equipped with a TurboIonSpray⁺ source. A hydroxide (NaOH) eluent at 0.3 mL · min⁻¹ was followed by 90% acetonitrile (0.3 mL · min⁻¹) as a postcolumn solvent. To overcome matrix effects, all samples were spiked with Cl^{18}O_3 (produced in-house at Texas Tech) or Cl^{18}O_4 (Dionex) internal standards.

The methods for solid phase microextraction (SPME) [Pawliszyn, 1999] and GC-MS were modified from that described in Niki *et al.* [2004] and Jaraula *et al.* [2008]. For the LVBr, a 2 mL aliquot of brine was pipetted into headspace sampling vials, which were previously baked at 500°C for 12 h. While the samples were continuously stirred, after 30 min, the liquid phase was sampled by direct immersion (DI) using a so-called “black” fused silica fiber coated with 75 μm of Carboxen[®]/polydimethylsiloxane (PDMS) phase (Supelco) or a “blue” fused silica fiber coated with 65 μm of PDMS/divinylbenzene (DVB) phase (Supelco). These SPME fibers were chosen because of their versatility, their comparable selectivity, and importantly because they have different organic phase coatings. These differences in composition provide a mean for easy distinction of the contribution of SPME phases to the volatiles analyzed.

Prior to use, fibers were activated for 30 min at 300°C (black fiber) and 250°C (blue fiber) using the split/splitless injector of a Hewlett Packard (HP) 5890 gas chromatograph (GC). For the DI sampling, the SPME fibers were held for 40 min at 40 ± 0.5°C in the liquid phase and transferred immediately to the injector of the GC.

The Carboxen[®] of the black SPME fiber corresponds to Carboxen[®] 1006, a solid polymeric, porous, synthetic carbon molecular sieve dominated by sp³ bonds with an even distribution of micropore (7 Å), mesopore, and macropore [Mani, 1999]. Carbon molecular sieve polymers are produced by pyrolysis of organic polymers [Jochmann *et al.*, 2014]. The Carboxen[®] 1006 and the DVB are embedded into, and partially cross-linked to, the liquid PDMS polymer. All open solvent bottles were removed from the laboratory at least 16 h prior to SPME use in order to prevent contamination of the laboratory atmosphere.

For GC-MS, a HP 6890 GC coupled to a HP-5973 mass selective detector was used in electron ionization mode at 70 eV with helium (Ultra High Purity, He 5.0, 99.999% from Praxair) as carrier gas in constant flow at 1.1 mL · min⁻¹. The column was a 30 m long HP-5MS (0.25 mm I.D., 0.25 μm film thickness; (5%-phenyl)-PDMS). An HP-5MS was selected for its versatility as it provides good chromatography for both polar and apolar compounds. The range of the mass scan was m/z 28 to 550 at a rate of three scans per second. Analytes were desorbed from the SPME fiber for 1 min into the injector, which was operated in splitless mode at 250°C for the blue fiber and at 300°C for the black fiber. These desorption temperatures are the maximum temperatures of use suggested by Supelco, the manufacturer of these fibers. The oven temperature was kept at 35°C for 2 min, then ramped at 1°C · min⁻¹ to 100°C and subsequently ramped at 10°C · min⁻¹ to 300°C · min⁻¹ and kept at 300°C for 30 min.

Analytical blanks correspond to the insertion of the blue or black SPME fibers into the split/splitless injector of the GC-MS directly after activation. A perchlorate reference sample with a concentration of 40 μg · L⁻¹ of ClO_4^- was also prepared by dissolving NaClO₄ (Sigma-Aldrich) into MilliQ water.

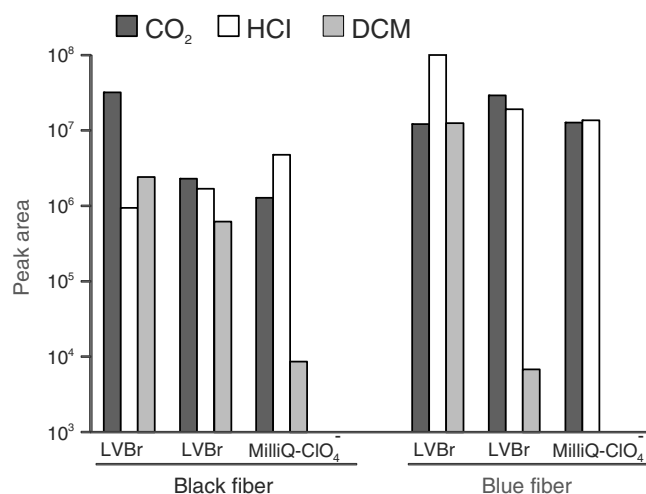


Figure 1. Peak area of CO₂ (black), HCl (white), and dichloromethane (DCM, grey) in the mass chromatogram of their most abundant fragment ion, m/z 44, m/z 36, and m/z 84, respectively, upon duplicate analysis of Lake Vida brine (LVBr) and MilliQ water with 40 $\mu\text{g} \cdot \text{L}^{-1}$ of perchlorate (MilliQ-ClO₄⁻) with DI-SPME GC-MS, using a black and a blue SPME fiber.

and black SPME fibers contained neither HCl nor DCM. Analytical blanks were devoid of organic compounds other than fragments of the column liquid phase such as hexamethyl-cyclotrisiloxane and noncyclic polysiloxanes. Note that these analytical blanks were obtained after the analysis of LVBr and the analysis of pure water blanks with ClO₄⁻, when the GC column phase and SPME fibers may have already suffered some damage.

DI-SPME GC-MS of MilliQ water spiked with ClO₄⁻ (40 $\mu\text{g} \cdot \text{L}^{-1}$) produced a significant amount of CO₂ and HCl with both black and blue fibers (Figure 1). In contrast, DCM was not detectable when using a blue fiber, while DCM detected when using a black fiber was 2 orders of magnitude less than what was detected when analyzing the DOC-rich LVBr. MilliQ water spiked with ClO₄⁻ yielded small amounts formic and acetic acids with both the black and blue fibers. Ketones (2-butanone and 2-pentanone), tetrahydrofuran, and ethyl acetate were observed only when using a black fiber (Table 1).

Analysis of the LVBr by DI-SPME GC-MS resulted in a total ion current trace dominated by the CO₂, DCM, and dimethylsulfide (DMS) (Figures 2 and S1 in the supporting information). CO₂ was observed in all brine samples and all blanks with perchlorates analyzed using both black and blue SPME fibers. Apart from CO₂, the compounds detected with both the blue and black fibers can be divided into five categories on the basis of their chemical composition: (i) Cl bearing, (ii) S and Se bearing, (iii) O bearing, (iv) saturated hydrocarbons, and (v) aromatic compounds (Figure 2 and Table 1).

1. Chloride-bearing compounds HCl and DCM were observed in all DI-SPME GC-MS analyses of LVBr. MeCl and TCM were observed, in low abundances, exclusively during analysis when using a black SPME fiber (Table 1). When using a blue SPME fiber, the peak area of HCl, monitored by the mass to charge ratio m/z 36, is 1 to 2 orders of magnitude larger than when using a black fiber (Figure 1).
2. The volatile organic sulfur compounds (VOSCs) identified upon DI-SPME GC-MS, include carbon disulfide (CS₂), DMS, dimethyl selenide (DMSe), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS). CS₂ was observed in LVBr when using a black SPME fiber (Table 1). DMS, DMSe, and DMDS were observed in all LVBr analysis, independent of the SPME fiber used (Table 1 and Figures 2 and S2). DMTS was observed only in one of two runs with the black fiber and in one of two runs with the blue fiber, in both cases in very low abundances. DMS is always the most abundant VOSC, followed, in decreasing order, by DMDS, DMSe, CS₂, and DMTS (Figure S2). VOSCs were neither observed in the analytical blanks nor upon analysis of MilliQ water with perchlorates. There is no source of sulfur in the SPME GC-MS system used, and both the analytical blanks and the SPME runs of MilliQ water with ClO₄⁻ did not produce any VOSCs, suggesting that all VOSCs species must be derived from the brine itself.
3. O-bearing organic compounds include formic, acetic, and propionic acids as well as ketones (2-propanone, 2-butanone, 2-pentanone, and 4-methylpentane-2-one; the latter being observed with both black

3. Results

3.1. Oxychlorines

The concentrations of perchlorate (ClO₄⁻) and chlorate (ClO₃⁻) in LVBr collected during the austral summer 2005–2006 are 49 and 11 $\mu\text{g} \cdot \text{L}^{-1}$, respectively. Lake Vida brine, the bottom waters of the West lobe of Lake Bonney, and the chemocline of Lake Fryxell (both Lake Bonney and Fryxell are in Taylor Valley; McMurdo Dry Valleys) are the only bodies of water of the dry valleys in which ClO₄⁻ is more abundant than ClO₃⁻ [Jackson *et al.*, 2012]. The concentration of chlorine in LVBr is $1.16 \cdot 10^5 \text{ mg} \cdot \text{L}^{-1}$ [Murray *et al.*, 2012].

3.2. DI-SPME GC-MS

Analytical blanks with both blue and black SPME fibers contained neither HCl nor DCM. Analytical blanks were devoid of organic compounds other than fragments of the column liquid phase such as hexamethyl-cyclotrisiloxane and noncyclic polysiloxanes. Note that these analytical blanks were obtained after the analysis of LVBr and the analysis of pure water blanks with ClO₄⁻, when the GC column phase and SPME fibers may have already suffered some damage.

DI-SPME GC-MS of MilliQ water spiked with ClO₄⁻ (40 $\mu\text{g} \cdot \text{L}^{-1}$) produced a significant amount of CO₂ and HCl with both black and blue fibers (Figure 1). In contrast, DCM was not detectable when using a blue fiber, while DCM detected when using a black fiber was 2 orders of magnitude less than what was detected when analyzing the DOC-rich LVBr. MilliQ water spiked with ClO₄⁻ yielded small amounts formic and acetic acids with both the black and blue fibers. Ketones (2-butanone and 2-pentanone), tetrahydrofuran, and ethyl acetate were observed only when using a black fiber (Table 1).

Analysis of the LVBr by DI-SPME GC-MS resulted in a total ion current trace dominated by the CO₂, DCM, and dimethylsulfide (DMS) (Figures 2 and S1 in the supporting information). CO₂ was observed in all brine samples and all blanks with perchlorates analyzed using both black and blue SPME fibers. Apart from CO₂, the compounds detected with both the blue and black fibers can be divided into five categories on the basis of their chemical composition: (i) Cl bearing, (ii) S and Se bearing, (iii) O bearing, (iv) saturated hydrocarbons, and (v) aromatic compounds (Figure 2 and Table 1).

1. Chloride-bearing compounds HCl and DCM were observed in all DI-SPME GC-MS analyses of LVBr. MeCl and TCM were observed, in low abundances, exclusively during analysis when using a black SPME fiber (Table 1). When using a blue SPME fiber, the peak area of HCl, monitored by the mass to charge ratio m/z 36, is 1 to 2 orders of magnitude larger than when using a black fiber (Figure 1).
2. The volatile organic sulfur compounds (VOSCs) identified upon DI-SPME GC-MS, include carbon disulfide (CS₂), DMS, dimethyl selenide (DMSe), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS). CS₂ was observed in LVBr when using a black SPME fiber (Table 1). DMS, DMSe, and DMDS were observed in all LVBr analysis, independent of the SPME fiber used (Table 1 and Figures 2 and S2). DMTS was observed only in one of two runs with the black fiber and in one of two runs with the blue fiber, in both cases in very low abundances. DMS is always the most abundant VOSC, followed, in decreasing order, by DMDS, DMSe, CS₂, and DMTS (Figure S2). VOSCs were neither observed in the analytical blanks nor upon analysis of MilliQ water with perchlorates. There is no source of sulfur in the SPME GC-MS system used, and both the analytical blanks and the SPME runs of MilliQ water with ClO₄⁻ did not produce any VOSCs, suggesting that all VOSCs species must be derived from the brine itself.
3. O-bearing organic compounds include formic, acetic, and propionic acids as well as ketones (2-propanone, 2-butanone, 2-pentanone, and 4-methylpentane-2-one; the latter being observed with both black

Table 1. Organic and Inorganic Compound Occurrence Observed upon DI-SPME GC-MS Duplicate Analyses of Lake Vida Brine (LVBr 1 and 2) and Analysis of MilliQ Water with $40 \mu\text{g ClO}_4^- \cdot \text{L}^{-1}$

| | Compounds | Black Fiber DI-SPME | | | Blue Fiber DI-SPME | | |
|-------------|------------------------------|---------------------|--------|------------------------------|--------------------|--------|------------------------------|
| | | LVBr 1 | LVBr 2 | MilliQ with ClO_4^- | LVBr 1 | LVBr 2 | MilliQ with ClO_4^- |
| | Carbon dioxide | x ^a | x | x | x | x | x |
| Cl | HCl | x | x | x | x | x | x |
| | Chloromethane | tr ^b | tr | | | | |
| | Dichloromethane | x | x | x | x | x | |
| | Trichloromethane | x | | | | | |
| S and Se | Dimethyl sulfide | x | x | | x | x | |
| | Dimethyl selenide | x | x | | x | tr | |
| | Dimethyl disulfide | x | x | | x | x | |
| | Dimethyl trisulfide | | x | | x | | |
| | Carbon disulfide | x | x | | | | |
| Hydrocarbon | Butene | x | tr | | | | |
| | Pentane | tr | | | | | |
| | 2-methylpentane | x | | | | | |
| | 3-methylpentane | x | | | | x | |
| | <i>n</i> -hexane | x | x | | | | |
| | Methylcyclopentane | x | | | | | |
| Aromatic | Benzene | x | x | | x | x | |
| | Toluene | x | | | x | x | |
| | Styrene | | | | x | | |
| | Ethylbenzene | | | | | x | |
| Oxygen | Formic acid | | x | x | tr | x | x |
| | Acetic acid | x | x | x | x | x | x |
| | Propionic acid | | | | | x | |
| | Ethanol | x | x | | | | |
| | Diethyl ether | x | | | | | |
| | 2-propanone | x | x | x | | | |
| | 2-butanone | x | x | x | x | | |
| | 2-pentanone | x | x | | | | |
| | 4-methyl-penta-2-one | x | x | | x | x | |
| | Ethyl acetate | x | x | x | | | |
| | Tetrahydrofuran | x | | x | | | |
| Si | Phenylsilane | x | x | | x | | |
| | Hexamethylcyclotrisiloxane | x | x | x | x | x | x |
| | Octamethylcyclotetrasiloxane | x | x | | x | | |

^aCross indicates compounds with unambiguous mass spectrum.
^btr indicates compounds in low abundance.

and blue fibers; Table 1 and Figure 2). In contrast, some compounds such as ethanol, diethyl ether, ethyl acetate, and tetrahydrofuran were only observed when a black SPME fiber was used. The DI-SPME GC-MS of MilliQ water with perchlorate (with both black and blue SPME fibers) contains formic acid and acetic acid. Propionic acid was only observed in minute amounts in one of the LVBr analysis with a blue SPME fiber. Some of the ketones (2-propanone and 2-butanone), as well as ethyl acetate and tetrahydrofuran, were observed in the MilliQ water with perchlorate analyzed with a black SPME fiber but were not observed in the MilliQ water with perchlorates when using a blue SPME fiber.

4. C₄, C₅, and C₆ hydrocarbons were identified (Table 1). With the exception of 3-methylpentadecane that was observed when LVBr was analyzed using a blue SPME fiber, all hydrocarbons were observed when a black fiber was used. Butane and *n*-pentane are present in small to trace abundances. C₆ hydrocarbons are dominated by *n*-hexane with 2- and 3-methylpentane as well as methylcyclopentane (Figure 2). These hydrocarbons were observed neither during analysis of MilliQ water with perchlorates nor in analytical blanks.
5. The aromatic compounds benzene and toluene were observed in DI-SPME GC-MS analysis of LVBr with both black and blue SPME fibers. In contrast, C₂-benzene styrene and ethylbenzene were observed exclusively when analyzing LVBr with a blue SPME fiber. Aromatic compounds were absent in the MilliQ water with perchlorate (Table 1) and in the analytical blanks.

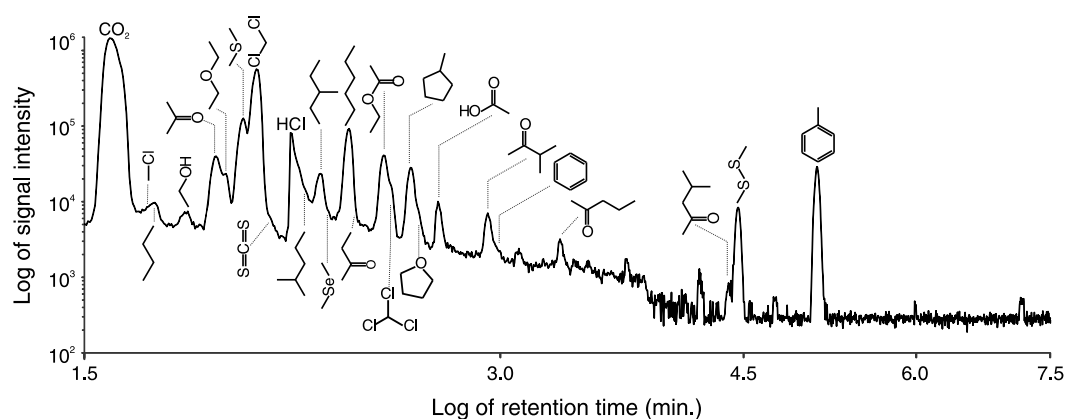


Figure 2. Log plot of the total ion current (TIC) trace obtained upon DI-SPME GC-MS with a black SPME fiber of Lake Vida brine. To show on the same plot the most and the least abundant peaks, the signal intensity is plotted on a log scale. To better observe the dense distribution of peaks between 1.5 and 3 min, the retention time was also plotted on a log scale. The corresponding nonlog TIC trace is shown in Figure S1.

4. Discussion

4.1. Oxychlorines

Oxychlorines in the lakes of the MDVs derive from atmospheric deposition on land as well as glaciers and are transported via ephemeral summer streams to closed basins [Jackson *et al.*, 2012]. In the MDVs, ClO_4^- and ClO_3^- concentrations were measured in soils and ice by Kounaves *et al.* [2010] and measured in ephemeral streams as well as surficial and deep waters of perennially ice-covered lakes by Jackson *et al.* [2012]. Jackson *et al.* [2012] showed that, in lakes of the dry valleys, the $\text{ClO}_3^-/\text{ClO}_4^-$ ratio is mostly constant with a value of 3.1 and proposed that values below 3.1 reflect the biodegradation of ClO_3^- in anoxic environments. The $\text{ClO}_3^-/\text{ClO}_4^-$ ratio for LVBr is 0.2, suggesting microbial degradation of ClO_3^- or abiotic degradation perhaps similar to that of NO_3^- observed experimentally in LVBr by Ostrom *et al.* [2016].

The $\text{ClO}_4^-/\text{Cl}^-$ and $\text{ClO}_3^-/\text{Cl}^-$ molar ratios of LVBr ($1.5 \cdot 10^{-7}$ and $5.3 \cdot 10^{-8}$, respectively) fall in the range of values measured for other dry valley lakes (10^{-7} – 10^{-9}); all of which have experienced significant degradation of ClO_4^- and ClO_3^- , presumably due to biological reduction [Jackson *et al.*, 2012]. However, the relative contribution of biological and abiotic reduction of oxychlorine cannot be resolved as this point.

LVBr is anoxic [Murray *et al.*, 2012] and, as such, could support microbial reduction of ClO_4^- and ClO_3^- . The reduction potential of ClO_4^- and ClO_3^- makes them ideal electron acceptors [Coates and Achenbach, 2004], and oxychlorine-reducing capacities seem to be widely distributed among bacteria [Coates *et al.*, 1999]. Thus, one can wonder why ClO_4^- and ClO_3^- are still so abundant in the anoxic LVBr. The answer could be related to electron acceptor competition with the abundant NO_3^- (nearly millimolar [Murray *et al.*, 2012]) in LVBr [Farhan and Hatzinger, 2009; London *et al.*, 2011; Ricardo *et al.*, 2012], although some organisms also reduce ClO_4^- and ClO_3^- in the presence of NO_3^- [Nozawa-Inoue *et al.*, 2005; Van Ginkel *et al.*, 2010; Zuo *et al.*, 2009]. In addition, very low microbial metabolic rates imposed by the very low temperature of LVBr (-13.4°C), with a calculated cell doubling time of 120 years [Murray *et al.*, 2012], would also prevent oxychlorines from being depleted by bacterial reduction even after millennia.

Oxychlorines are formed in the atmosphere [Catling *et al.*, 2010; Kounaves *et al.*, 2010], and the presence of these compounds in an encapsulated brine must represent a legacy of a former Lake Vida that received light and external inputs. The history of Lake Vida prior to and during brine formation is not known well enough [Dugan *et al.*, 2015b] to exclude the possibility that the $\text{ClO}_3^-/\text{ClO}_4^-$ ratio was fixed prior to brine encapsulation. This could, for example, occur during periods of lake water column stratification with anoxic bottom waters, as observed in other dry valley lakes such as Lake Fryxell (Taylor Valley [Priscu, 1998]). It is also conceivable that a former Lake Vida with oxygenated waters would also prevent microbial reduction, although the timing of when this system was oxygenated is currently unknown.

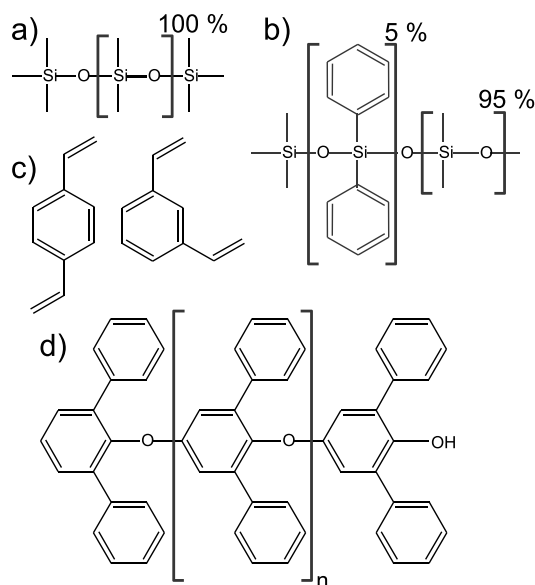


Figure 3. (a) Structure of polydimethylsiloxane (PDMS). (b) Structure of (5%-phenyl)-PDMS used as a liquid phase in the capillary column. (c) Isomers of divinylbenzene in blue SPME fibers. (d) Structure of Tenax TA used on SAM.

4.2. CO₂, HCl, and Chlorinated Organic Compounds

In our experiments, CO₂ is derived from the oxidation of carbon by the oxygen released from oxychlorines in the 250°C (blue phase) and 300°C (black phase) split/splitless injector of the GC, as is observed in the pyrolysis experiments mimicking SAM and in actual SAM experiments on Mars [Glavin *et al.*, 2013]. CO₂ is present in MilliQ water with ClO₄[−] when using both Carboxen®/PDMS and PDMS/DVB SPME phases (Figure 3). In these experiments, the only sources of carbon are the coating of SPME fibers and the liquid stationary phase coating of the capillary column (5%-phenyl-PDMS; Figure 3). Thus, the CO₂ produced during analysis of LVBr must be derived from both the oxidation of compounds original to

the brine and oxidation of the organic phases of the SPME GC-MS system. SPME analysis of organic compounds can be influenced by the presence of Cl[−] on the SPME fibers, where it competes for adsorption sites with other compounds. However, the abundance of HCl produced during analysis of MilliQ water with ClO₄[−] is equivalent to the amount produced during analysis of LVBr with both the blue and black SPME fibers (Figure 1). Thus, the Cl[−] adsorbed on the SPME fibers during DI of LVBr does not significantly influence production of HCl during SPME GC-MS. Thus, the chlorine of HCl is provided mostly by the breakdown of oxychlorines. As helium is used as a carrier gas, it does not contribute to the hydrogen of HCl. The hydrogen could be derived from residual water remaining on the SPME fibers after DI, from the organic phases of the SPME fibers or the chromatographic column, or from organic compounds adsorbed on the fibers when running a sample of LVBr. There are no significant differences in HCl abundance between LVBr samples and MilliQ water with ClO₄[−] run (Figure 1), suggesting that the organic matter in LVBr is not necessary to provide enough hydrogen to the system to form the HCl observed. The source of hydrogen must then be either residual water or the organic phase of the SPME GC-MS system or both. HCl was not observed among the volatiles released upon headspace (HS)-SPME GC-MS of LVBr when using a black fiber (Figure S1). In HS-SPME, the fiber is not in direct contact with the liquid sample, suggesting that residual water on the fiber is the likely source of the hydrogen of HCl.

DCM is present in all LVBr samples analyzed, independent of the SPME fiber used. In contrast, when the MilliQ water with ClO₄[−] is analyzed with a black SPME fiber, DCM is present in low abundance or absent relative to DCM in the volatiles of LVBr samples when analyzed with the same fibers. When analyzing MilliQ water with ClO₄[−], the reactions associated with the decomposition of ClO₄[−] in the GC injector are carbon limited. The sources of carbon, the SPME fiber coatings and the liquid phase of the capillary column, do not provide enough carbon for DCM to be abundant or form at all. In contrast, the carbon provided by LVBr allowed ample formation of DCM.

4.3. Volatile Sulfur and Selenium Compounds

The legacy of a prior photosynthetic ecosystem is well illustrated in LVBr by the presence of the VOSCs (CS₂, DMS, DMDS, DMTS, and DMSe) in the volatiles evolved from LVBr upon DI-SPME GC-MS (Figures 2 and S2 and Table 1). DMS is a product of bacterial catabolism of dimethylsulfoniopropionate (DMSP) [Reisch *et al.*, 2011; Stefels and van Boekel, 1993], a compound produced by photosynthetic algae in marine and lacustrine environments. DMDS and DMTS sources are directly related to those of DMS. DMSe is a product of microbial catabolism of selenoproteins produced by algae including diatoms and prymnesiophytes [Araie and Shiraiwa, 2009], algal phyla that are present in lakes of the dry valleys [Jaraula *et al.*, 2010; Spaulding *et al.*, 1997].

Photosynthesis does not presently occur in LVBr [Murray *et al.*, 2012] but must have occurred in the former Lake Vida when it received light and external inputs, prior to brine cryoencapsulation. Diatom frustules were detected in lower sediment layers of the Lake Vida ice core [Dugan *et al.*, 2015b]. The presence of DMS, DMDS, DMTS, and DMSe is well known in marine environments including sea ice [Levasseur, 2013; Spiess *et al.*, 2009; Turner *et al.*, 1995] but is also observed in both saline and freshwater lakes, including Antarctic lakes [Gibson *et al.*, 1991; Lee *et al.*, 2004; Roberts and Burton, 1993].

The quantitative assessment of VOSCs by Murray *et al.* [2012] provided the concentrations of DMS, MeSH, and dimethylsulfoxide (DMSO; 0.1, 0.2, and 25 $\mu\text{mol}\cdot\text{L}^{-1}$, respectively). DMSO and dimethylsulfone (DMSO₂), common oxidation products of DMS, were not detected in LVBr by DI-SPME GC-MS. The SPME fibers used here are not appropriate to collect these relatively high-boiling-point, high-polarity compounds [e.g., Camarasu, 2000]. Thus, the absence of DMSO and DMSO₂ from the VOSCs observed suggests that the oxidative power associated with breakup of oxychlorine in the injector of the GC did not result in oxidation of VOSCs. It is noteworthy that DMSO₂ is present in the liquid/liquid total extract of the very same samples of LVBr used here. The reasons why the VOSCs did not get oxidized remain unclear at this point.

MeSH is also a product of microbial catabolism of DMSP via demethylation [Levasseur, 2013]. The absence of MeSH upon SPME analysis must be reconciled with the measurements of Murray *et al.* [2012] that show that MeSH (0.2 $\mu\text{mol}\cdot\text{L}^{-1}$) is more abundant than DMS (0.1 $\mu\text{mol}\cdot\text{L}^{-1}$) in LVBr. In a study of artifact formed upon black fiber SPME analysis of VOSCs, Lestremau *et al.* [2004] show that mercaptans like MeSH react to form their corresponding dimers, explaining the presence of DMDS and the nondetection of MeSH in all LVBr analysis. A contributing factor to the absence of MeSH is a probable partial loss of this very volatile compound during transfer of LVBr to SPME vials.

DMSP was not detected with our SPME GC-MS methods, although a black fiber was used previously for quantification of DMSP by SPME GC-MS [Niki *et al.*, 2004]. Hence, the absence of DMSP cannot be ascribed to an analytical deficiency. DMSP is usually quickly turned into DMS via enzymatic processes [Stefels *et al.*, 2007]. This degradation most likely occurred prior to the encapsulation of the brine.

Although the relative abundance of VOSCs observed upon SPME GC-MS may have been influenced by technique-specific artifacts, all the VOSCs or their source compounds derive from LVBr.

4.4. Aromatics

Aromatic compounds identified in the volatiles of LVBr (benzene, toluene, styrene, ethylbenzene, and phenylsilane) could be evolved from the brine itself but could also be degradation products of organic polymers involved in the SPME GC-MS system. Benzene and phenylsilane could be derived directly from the breakdown of the liquid phase of the 5%-phenyl-PDMS capillary column used (Figure 3). Benzene can also be derived from Carboxen[®] as it was observed in SPME GC-MS blank analysis using a black fiber by Lestremau *et al.* [2004], although we did not detect benzene in our analytical blanks. Toluene, styrene, and ethylbenzene cannot be formed directly from the phenyl substituent of 5%-phenyl-PDMS phase as benzene is a substituent of the silicon atom of siloxane. Styrene and ethylbenzene are not present in the volatiles of LVBr when analyzed using a black SPME fiber made of Carboxen[®] and PDMS. In contrast, styrene and ethylbenzene are present in the volatiles of LVBr analyzed using a blue SPME fiber with a phase made of PDMS and DVB (Table 1 and Figure 3). The DVB of the blue SPME fiber is most likely the source of the toluene and styrene observed, explaining why styrene and ethylbenzene are not observed in experiments using a black SPME fiber. However, the presence of a toluene peak in one of the LVBr DI-SPME GC-MS using a black SPME fiber cannot be explained directly by contribution of GC column and SPME phases involved, unless Carboxen[®], which composition is not well known, can also release toluene. As a result, the presence of benzene and toluene in LVBr cannot be excluded at this point, but the presence of styrene and ethylbenzene is related to the DVB of the blue SPME fiber.

Thus, the absence of aromatic compounds upon black and blue fiber DI-SPME GC-MS of MilliQ water with ClO_4^- (Table 1) does not necessarily indicate that these compounds are derived from LVBr. It is most likely that all aromatics observed, at the exception of benzene and toluene which may be derived from LVBr, are artifacts derived from the SPME phases. As indicated above, the low abundance or absence of DCM in the compounds produced upon DI-SPME GC-MS of MilliQ water with ClO_4^- suggests that the reactions in the GC injector are carbon limited. It can be hypothesized that the aromatic compounds released from the SPME fibers are not observed upon analysis of MilliQ water with ClO_4^- because these compounds are fully oxidized to CO_2 by the by-products of decomposition of oxychlorines.

4.5. Oxygenates

Representatives of the major species of oxygenates (ketones and acids) observed upon DI-SPME GC-MS of LVBr were also observed in the DI-SPMDE GC-MS of the MilliQ water with ClO_4^- , though in lesser abundance relative to CO_2 and HCl. These lower abundances and lesser diversity support the carbon-limited reaction hypothesis proposed above to explain the low abundance of DCM and absence of aromatic compounds upon DI-SPME GC-MS of MilliQ water with ClO_4^- . It is noteworthy that tetrahydrofuran was observed in the SPME GC-MS blank analysis using a black fiber by *Lestremau et al.* [2004], explaining why it is only observed when using a black SPME fiber. It can then be speculated that only some of the oxygen-bearing organic compounds formed as a result of carbon limitation. The presence of abundant DOC in LVBr allowed for the formation of a more diverse array of oxygen-bearing compounds; most if not all being artifacts formed in the injector of the GC upon decomposition of oxychlorines or like tetrahydrofuran directly derived from the SPME phase.

4.6. Hydrocarbons

Determining the origin of hydrocarbons observed during DI-SPME GC-MS of LVBr is complex. The absence of hydrocarbon from the analytical blank and MilliQ water with ClO_4^- may indicate that these compounds are not directly derived from the organic phases used in the SPME GC-MS system. It also means that these compounds may not form from the reaction of the by-products of oxychlorine degradation with the SPME fiber in the GC injector. However, the MilliQ blank with ClO_4^- is completely depleted in carbon, and therefore, it is likely that any hydrocarbon derived from the SPME fibers upon reaction with ClO_4^- degradation products would be oxidized to form CO_2 . As a result, we cannot determine if these hydrocarbons are actually derived from LVBr or if they are artifacts produced in the injector and are protected (in part) from degradation by organic compounds provided by LVBr. Last but not least, a possible contamination by the laboratory atmosphere must be considered. All the C_6 hydrocarbons could be derived from the laboratory hexanes lot, even if chromatographic tests of our lots of hexanes show a very different distribution, dominated by cyclohexane and *n*-hexane, but with neither 2- nor 3-methylpentanes.

4.7. Carbon Limitation

The low amount of organic carbon (SPME fiber coating and GC column liquid phase) available for reaction in the GC injector upon DI-SPME GC-MS of MilliQ water with ClO_4^- seems to be a major controlling factor of the distribution of organic compounds in the blank. This hypothesis is supported by the low abundance or absence of DCM, the absence of aromatic compounds, and low abundance of oxygenated compounds upon DI-SPME GC-MS of MilliQ water with ClO_4^- . The absence of DCM in the volatiles of MilliQ water with ClO_4^- when analyzed with a blue SPME fiber is not an indication that the DCM observed upon analysis of LVBr is present in the brine itself. Similarly, the absence of aromatic compounds among the molecules observed during the DI-SPME GC-MS of MilliQ water with ClO_4^- is not an indication that all aromatic compounds observed upon DI-SPME GC-MS of LVBr are actually derived from LVBr. The correlation between the distribution of aromatic compounds and the type of SPME fiber used in our experiments suggests that some of these aromatic compounds (ethylbenzene and vinylbenzene) are artifacts, produced by the breakdown of the coating of the SPME fibers. Thus, a carbon-limited blank such as DI-SPME GC-MS of MilliQ water with ClO_4^- does not reflect the diversity of the competing reactions taking place in the injector of the GC upon decomposition of the ClO_4^- in the presence of instrument organic carbon. Furthermore, it seems that the by-products of the degradation of SPME fibers, not visible in the MilliQ water with ClO_4^- , are being oxidized to CO_2 or DCM. With our experimental setup, in order to observe the variety of artifacts formed in the injector by oxychlorine decomposition, a well-defined source of organic carbon should be added to the MilliQ water with ClO_4^- .

Apart from the VOSCs, no compounds unambiguously derived from LVBr were observed upon SPME GC-MS, suggesting that reactions between oxychlorine decomposition by-products and brine compounds were also carbon limited. LVBr has a DOC concentration $48.2 \pm 9.7 \text{ mmol} \cdot \text{L}^{-1}$, largely in excess of the molar abundance of perchlorate and chlorate (0.49 and $0.11 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$, respectively), but a vast majority of the DOC in LVBr is not amenable to DI-SPME as Carboxen®/PDMS (black) and PDMS/VDB (blue) fibers were designed for molecules in the C_2 – C_{12} range [*Mani*, 1999]. Around 50% of the DOC of LVBr are in molecules larger than 1 kDa [*Cawley et al.*, 2016]. Two other fractions of dissolved organic matter (DOM), representing 18% of DOC, have compounds with an average molecular weight of ~ 500 Da, as determined by Fourier transform ion cyclotron resonance

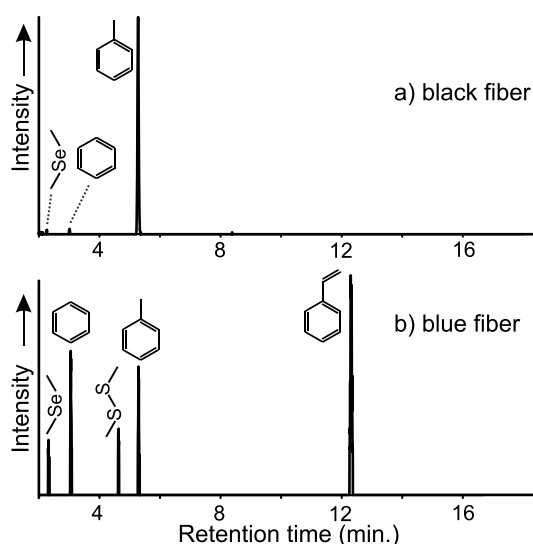


Figure 4. Summed mass chromatogram m/z 78 + m/z 91 + m/z 92 + m/z 103 + m/z 104 + m/z 105 + m/z 106 showing the distribution of benzene (m/z 78), toluene (m/z 91 + m/z 92), ethylbenzene (m/z 105 + m/z 106), and styrene (m/z 103 + m/z 104) in the volatiles observed upon DI-SPME GC-MS of LVBr when using (a) a black SPME fiber and (b) a blue SPME fiber. Note that ethylbenzene was not detected in this run of LVBr using a blue fiber.

products of the heat-induced decomposition of oxychlorines during analysis of LVBr samples by SPME GC-MS. The same applies for the sediment samples analyzed by SAM at Rocknest [Glavin *et al.*, 2013; Leshin *et al.*, 2013] and at Yellowknife Bay [Freissinet *et al.*, 2015; Ming *et al.*, 2014]. The origin of the chlorine in HCl, MeCl, DCM, and TCM is not controversial as all authors cited above agree that Cl derives mostly, if not all, from oxychlorines. In contrast, the origin of the carbon in CO₂, MeCl, DCM, TCM, and other chlorinated compounds is a more complex issue. In our DI-SPME GC-MS analysis of MilliQ water with ClO₄⁻, the only source of C is the organic phases of the SPME fibers and the capillary column. For blank analyses on SAM, carbon sources include Tenax TA, an absorbent used to concentrate organic compounds in a hydrocarbon trap prior to chromatographic analysis, and MTBSTFA, a derivatization agent that unfortunately leaked from one of its sealed capsules [Glavin *et al.*, 2013; Leshin *et al.*, 2013]. The small amount of compounds observed in the evolved gas analysis (EGA)-Tenax trap-GC-MS instrument blanks and in Rocknest samples were all associated to these sources of carbon [Glavin *et al.*, 2013; Leshin *et al.*, 2013]. For example, the aromatic compounds detected by SAM at Rocknest (benzene, toluene, phenylethyne, chlorobenzene, styrene, and biphenyl) were among volatiles observed in both instrument blanks and sediment samples [Leshin *et al.*, 2013]. The source of all these aromatic compounds is the degradation of Tenax TA [Glavin *et al.*, 2013], which is a porous polymer of 2,6-diphenylphenylene oxide (Figure 3). These compounds form in the Tenax cryotrap (kept at -10°C) and/or during heating of the Tenax trap to 300°C for the release of the trapped compounds to SAM's GC-MS (Figure 4).

Laboratory analog experiments described by Glavin *et al.* [2013] and Freissinet *et al.* [2015] used MTBSTFA ($12.04 \cdot 10^{-3}$ mol of C) and dimethyl formamide (DMF; $3.87 \cdot 10^{-3}$ mol of C) mixed with 28 mg of Ca(ClO₄)₂ · nH₂O. The amount of carbon in this experiment exceeds by 1 order of magnitude the potential amount of O₂ ($<2.11 \cdot 10^{-4}$ mol) and Cl₂ ($<1.17 \cdot 10^{-4}$ mol) produced upon decomposition of the hydrated Ca perchlorate. The identification of large amounts of MTBSTFA-derived compounds upon GC-MS [Freissinet *et al.*, 2015; Glavin *et al.*, 2013] is not unexpected, as only a small portion of the MTBSTFA carbon was used for synthesis of CO₂ and C₁ chlorinated compounds (MeCl, DCM, and TCM). Note that for the calculations of molar abundances of O₂ and Cl₂, as the hydration level of the Ca perchlorate used for these experiments was not provided, we considered the mass of Ca perchlorate as dry, thus exaggerating the molar amount of O₂ and Cl₂ produced upon degradation of the perchlorate hydrates by 23% if Ca(ClO₄)₂ · 4H₂O was used. With a 23% decrease in the molar abundance of O₂ and Cl₂ produced upon decomposition of Ca(ClO₄)₂ · 4H₂O, the above experiment is still not carbon limited.

mass spectrometry [Cawley *et al.*, 2016]. As a result of the very limited carbon range of the SPME fibers and the large size of the constituents of the DOM of LVBr, the reduced-carbon to oxychlorine ratio of the material adsorbed on the SPME fibers after DI sampling must have been much less than in the brine itself, although it cannot be quantified at this point. The reaction between oxychlorine decomposition by-products and brine compounds were carbon limited but not enough to prevent the expression of artifacts such as aromatic compounds and oxygenates as well as prevent the expression of VOSCs.

4.8. Implications for Martian Organic Matter

The HCl and chlorinated compounds with one carbon atom (MeCl, DCM, and TCM) are by-

Another analog experiment was performed using 1 mg of the kerogen-like organic matter of the Murchinson meteorite with 500 μg of Ca perchlorate. Again, this experiment was not carbon limited, with $5.65 \cdot 10^{-5}$ mol of carbon for a potential of $<2.00 \cdot 10^{-6}$ mol of Cl_2 and $<8.36 \cdot 10^{-6}$ mol of O_2 . Thus, it is not surprising that compounds expected from the pyrolysis of the kerogen of the Murchinson meteorite were observed, even if others, such as the more labile *n*-alkylthiophenes and *n*-alkylbenzenes, were altered [Freissinet *et al.*, 2015].

In contrast, some of the analog experiments described in Freissinet *et al.* [2015] were carbon limited. For example, to test whether the chlorobenzene can be formed from aromatic species, $0.65 \cdot 10^{-9}$ mol of benzene and $47 \cdot 10^{-9}$ mol of toluene were pyrolyzed separately in a SAM analog setup with 50 μg of Ca perchlorate. Upon pyrolysis, the Ca perchlorate would produce $<8.37 \cdot 10^{-7}$ mol of O_2 and $<2.09 \cdot 10^{-7}$ mol of Cl_2 . The benzene and toluene could provide only $3.90 \cdot 10^{-9}$ and $3.29 \cdot 10^{-7}$ mol of C, respectively, not enough carbon to uptake the O_2 and Cl_2 produced upon decomposition of the perchlorate. Thus, in absence of enough carbon to address the amount of O_2 and Cl_2 released by the Ca-perchlorate decomposition, as observed during our analysis of carbon-limited MilliQ water with ClO_4^- , it is likely that any organic compounds produced during EGA or pyrolysis (for analog experiments) and any by-product of Tenax TA degradation would be oxidized to CO_2 or C_1 chlorinated compounds and would not be visible in the produced chromatograms or be present in very low abundances. Thus, it is not surprising that chlorobenzene was “below background level” during GC-MS analysis [Freissinet *et al.*, 2015], for both benzene and toluene experiments. As a result, this analog experiment cannot be used to determine if the presence of aromatic compounds during analysis on Earth and on Mars influences the formation of chlorobenzene.

Similarly, on Mars, if the molar abundance of carbon in a sample is less than the molar abundance of O_2 and Cl_2 produced upon oxychlorine decomposition, it is likely that most, if not all, organic compounds derived from the sample will be oxidized during EGA to CO_2 or used as substrate for C_1 chlorinated compounds. Additionally, some of the oxidants (O_2 , Cl_2 , and HCl) could be transferred from the EGA to the Tenax trap, where they could, upon heating of the trap to 300°C , degrade the Tenax and Tenax by-products.

If the molar abundance of carbon in the sample is higher than the molar abundance of O_2 and Cl_2 available in the EGA, some of the organic compounds present may be preserved but organic artifacts produced in the analytical system (notably the Tenax trap) will also have a better chance to survive the effects of perchlorate decomposition, as we observed during our analysis of LVBr. Thus, it can be speculated that the increased abundance of these chlorinated compounds during analysis of the Sheepbed mudstone samples CB-3, CB-5, and CB-6 may be the result of an increase in the availability of Martian reduced carbon in the analyzed samples, allowing the expression of organic compounds otherwise oxidized to CO_2 or C_1 chlorides by the oxidants (O_2 and Cl_2) released upon perchlorate decomposition.

To increase the chances to have Martian organic matter expressed during EGA-trap-GC-MS, an additional source of reduced carbon could be added to the analyzed samples. On SAM, the only available additional sources of carbon are derivatization agents MTBSTFA and DMF stored in seven cups of the sample manipulation system. Although at least one of these cups leaked [Glavin *et al.*, 2013], each nonleaking derivatization cup contains 1.7 mmol of MTBSTFA and 1.3 mmol of DMF, which corresponds to 19.2 mmol of carbon. The abundance of perchlorates in Mars samples analyzed by SAM was estimated using the amount of O_2 released during EGA (from 0.1 to ~ 2 wt % ClO_4^- [Stern *et al.*, 2015; see also Ming *et al.*, 2014]). For a maximum sample size (triple load) of ~ 166 mg of sediment [Freissinet *et al.*, 2015], 2 wt % of ClO_4^- would provide $6.70 \cdot 10^{-2}$ mmol of O_2 and $1.67 \cdot 10^{-2}$ mmol of Cl_2 , much less than the moles of carbon provided by MTBSTFA and DMF. In such a case, the O_2 and Cl_2 released upon decomposition of the perchlorates would oxidize part of the available Martian reduced carbon and part of the MTBSTFA and DMF, allowing some Martian organic matter to be preserved and analyzed by GC-MS.

5. Conclusions

The cryoencapsulated, aphotic, anoxic LVBr contains a significant amount of perchlorate ($49 \mu\text{g} \cdot \text{L}^{-1}$) and chlorate ($11 \mu\text{g} \cdot \text{L}^{-1}$). The persistence of these oxychlorines in the anoxic brine is probably the result of the extremely low metabolic rates of the LVBr bacterial community and the inhibition of oxychlorine reduction by the presence of abundant nitrate.

Analysis of volatiles from the LVBr by DI-SPME GC-MS using two different SPME fibers showed that VOSCs are derived from LVBr and do not seem to be affected by oxychlorines upon analysis. These VOSCs are derived from

bacterial catabolism of DMSPs that are products of photosynthesis and, like oxychlorines that are products of atmospheric photochemistry, are legacies from a former Lake Vida that received light and external inputs.

LVBBr is a good Mars analog for brine that may have been preserved under ice or deep in lacustrine sediments. The presence of oxychlorines in LVBBr also suggests that perchlorates could be pervasive in any evaporative brine and lake sediments on Mars.

The analysis by DI-SPME GC-MS of MilliQ water with ClO_4^- did not produce any organic compounds other than DCM and oxygen-bearing compounds, and produced much less of those than during LVBBr analysis, suggesting carbon limitation of the experimental system. The composition of aromatic compounds observed upon analysis of LVBBr is in part dependent on the type of SPME fiber used during analysis, suggesting that some of these aromatic compounds may be artifacts. The absence of aromatic compounds in the volatiles evolved upon analysis of carbon-limited MilliQ water with ClO_4^- indicates that these compounds must have been oxidized to CO_2 or used to make C_1 chlorinated compounds upon decomposition of the oxychlorines in the injector of the GC. Thus, carbon-limited perchlorate blanks may not provide any information on the potential formation of artifacts associated with oxychlorine decomposition. Our experiments with LVBBr suggest that these artifacts are observed only if enough carbon is available during decomposition of the oxychlorines, preventing their full oxidation to CO_2 or C_1 chlorides.

Analysis of results obtained on SAM analog experimental systems and obtained by SAM on Mars suggests that carbon limitation has not been considered an important factor and that some of the conclusions drawn on the basis of analog experiments may need to be reconsidered. The appearance of high abundances of chlorobenzene as well as C_2 , C_3 , and C_4 dichloroalkanes in Sheepbed mudstone samples CB-3, CB-5, and CB-6 relative to other samples analyzed and relative to analytical blanks may reflect an increase in reduced-carbon/perchlorate ratio of these samples relative to other Martian samples analyzed. A high reduced-carbon/perchlorate ratio may prevent full oxidation of Martian reduced carbon. We propose that the addition of the derivatization agents MTBSTFA and DMF to Martian sediment samples would provide enough reduced carbon to prevent full oxidation of the organic matter in a Martian sample during pyrolysis, perhaps allowing the detection of Martian organic compounds by GC-MS.

Acknowledgments

The GC-MS data can be made available upon request to F.K. We thank J. Kyne and B. Bergeron (Ice Coring and Drilling Services), B. Wagner (University of Cologne), B. Glazer (University of Hawaii), and P. Glenday for the field assistance. This work was supported in part by National Aeronautics and Space Administration (NASA)-ASTEP NAG5-12889 (to P.T.D.) and National Science Foundation (NSF) awards ANT-0739681 (to A.E.M. and C.F.) and ANT-0739698 (to P.T.D. and F.K.). In 2005, the NSF Office of Polar Programs provided logistical support through a cooperative agreement with NASA. L.C. was supported by the Illinois Space Consortium Graduate Fellowship.

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