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# Temporal dynamics of halogenated organic compounds in Marcellus Shale flowback

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1 **Temporal Dynamics of halogenated organic compounds in Marcellus Shale flowback**  
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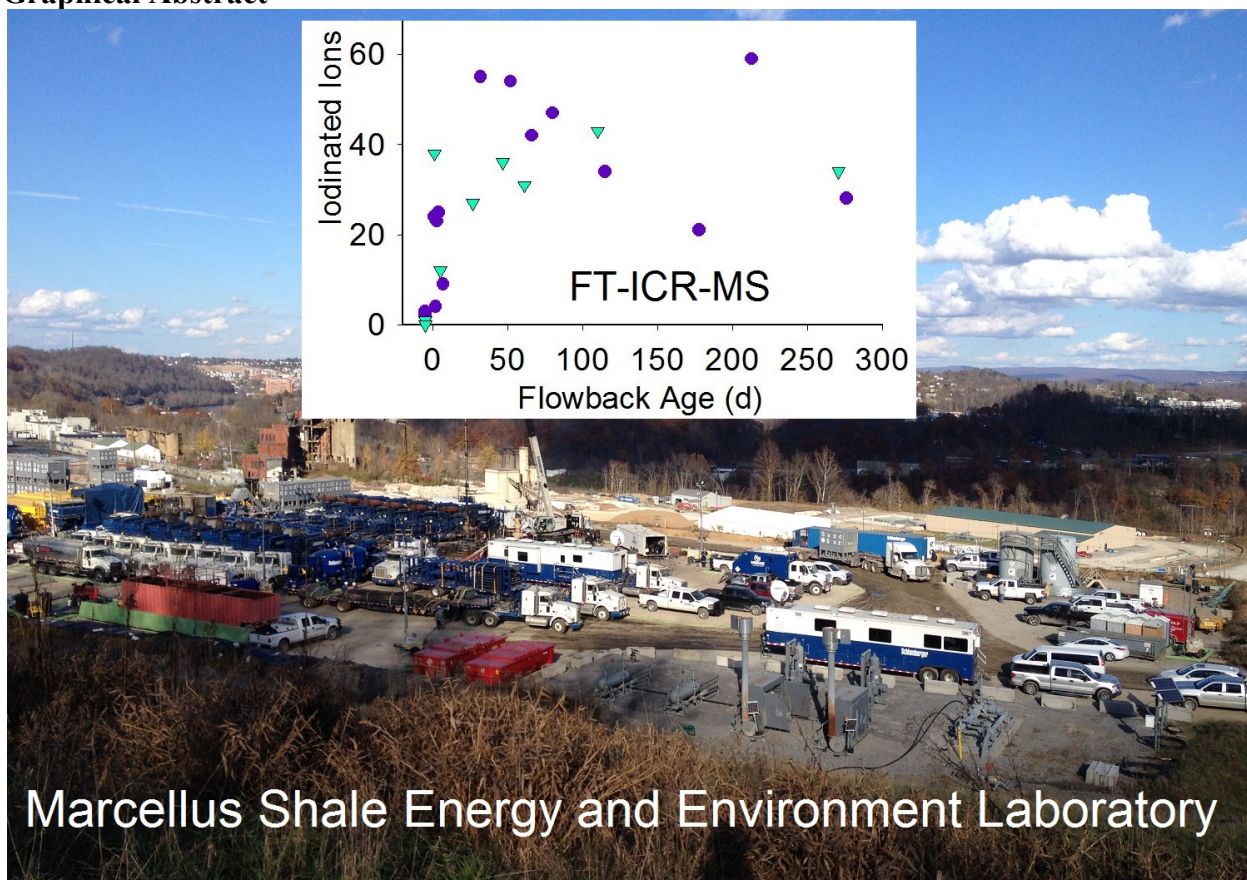
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16  
17 ***Key words: high volume hydraulic fracturing, Marcellus Shale, flowback fluid, produced***  
18 ***water, halogenated organic compounds***

19

19 **Graphical Abstract**



20  
21  
22

**Highlights**

- 23 • FT-ICR-MS reveals temporal changes in shale gas well organic chemical composition
- 24 • Many organohalogens in fluid were unique to the first 3 months of well operation
- 25 • Iodinated organic ions in fluid remain abundant over ten months of well operation
- 26 • Abiotic and biotic reactions may drive iodinated organic compound formation

27

27 **Abstract**

28           The chemistry of hydraulic fracturing fluids and wastewaters is complex and is known to  
29 vary by operator, geologic formation, and fluid age. A time series of hydraulic fracturing fluids,  
30 flowback fluids, and produced waters was collected from two adjacent Marcellus Shale gas wells  
31 for organic chemical composition analyses using ultrahigh resolution mass spectrometry.  
32 Hierarchical clustering was used to compare and extract ions related to different fluid ages and  
33 many halogenated organic molecular ions were identified in flowback fluids and early produced  
34 waters based on exact mass. Iodinated organic compounds were the dominant halogen class in  
35 these clusters and were nearly undetectable in hydraulic fracturing fluid prior to injection. The  
36 iodinated ions increased in flowback and remained elevated after ten months of well production.  
37 We suggest that these trends are mainly driven by dissolved organic matter reacting with reactive  
38 halogen species formed abiotically through oxidizing chemical additives applied to the well and  
39 biotically via iodide-oxidizing bacteria. Understanding the implications of these identified  
40 halogenated organic compounds will require future investigation in to their structures and  
41 environmental fate.

42

43 **1. Introduction**

44           Halogenated organic compounds (HOCs) have been recently identified in shale gas  
45 wastewaters using both targeted and non-targeted analytical approaches (Hoelzer et al., 2016;  
46 Luek et al., 2017; Maguire-Boyle and Barron, 2014). Furthermore, HOCs are of particular  
47 interest because they are not known additives used in the hydraulic fracturing process and  
48 although a handful of mechanisms have been proposed, their origin remains unknown (Hoelzer

49 et al., 2016; Luek et al., 2017; Maguire-Boyle and Barron, 2014). The environmental fate and  
50 toxicity of these compounds also remains unknown.

51 Non-targeted ultrahigh resolution Fourier transform ion cyclotron resonance mass  
52 spectrometry (FT-ICR-MS) is widely used to investigate the chemical composition of diverse  
53 organic matter based on its high mass accuracy and resolution (Dvorski et al., 2016; Gonsior et  
54 al., 2011; Kellerman et al., 2014; Roullier-Gall et al., 2015; Walker et al., 2014). This approach  
55 has been applied to complex natural and engineered systems to describe compositional changes  
56 not understood *a priori* and without the use of hundreds or thousands of costly analytical  
57 standards (Chen et al., 2016; Gonsior et al., 2016; Lavonen et al., 2013; Shakeri Yekta et al.,  
58 2012; Sleighter et al., 2014). Ultrahigh resolution FT-ICR-MS operated in negative mode is an  
59 appropriate approach for identifying unknown deprotonated HOCs including diverse disinfection  
60 by-products (DBPs) (Gonsior et al., 2015; Lavonen et al., 2013; Luek et al., 2017; Xu et al.,  
61 2013). Paired with solid phase extraction (Dittmar et al., 2008), FT-ICR-MS is uniquely suited  
62 for describing the temporal dynamics of diverse HOCs found in high salinity shale gas  
63 wastewater.

64 Understanding the behavior of HOCs within an individual hydraulic fracturing well is  
65 essential for narrowing down their possible origins and environmental fate. HOCs have been  
66 hypothesized to be transformation products (Hoelzer et al., 2016; Luek et al., 2017), but time  
67 series data have not been used previously to investigate this possibility. Therefore, the aim of this  
68 study was to track changes to the dissolved organic matter (DOM) pool of Marcellus Shale gas  
69 well fluids over the first ten months of well operation. Specifically, we combined solid phase  
70 extraction with FT-ICR-MS and used hierarchical clustering analysis to identify key shifts in the  
71 distribution of HOCs and suggest plausible formation mechanisms.

72

## 73 **2. Methods**

74 Hydraulic fracturing fluid, flowback fluid, and produced water samples were collected  
75 from two adjacent hydraulic fracturing wells at the Marcellus Shale Energy and Environment  
76 Laboratory (MSEEL) field site in Morgantown, WV (**Figure S1**) between November 2015 and  
77 September 2016 (Carr, 2017). DOM was solid phase extracted from water samples and analyzed  
78 using FT-ICR-MS. Hierarchical clustering analyses were performed on the resulting ions and  
79 assigned molecular formulas to track the temporal trends of HOCs.

### 80 *2.1 Sample Collection*

81 MSEEL wells MIP-3H and MIP-5H (herein referred to as 3H and 5H) were sampled  
82 from a gas-fluid separator in autoclaved high-density polyethylene carboys from the separator  
83 outlet. Fluid was then transferred in to 1L base-washed low-density polyethylene containers  
84 using a peristaltic pump, minimizing headspace. Samples were collected approximately daily  
85 during the initial week of flowback (December 2015, fluid production rates as high as 1000  
86 barrels d<sup>-1</sup>), bi-weekly for the following 3 months (early production water, fluid production rates  
87 10s of barrels d<sup>-1</sup>), monthly for 3 months, and then bimonthly (late production water, fluid  
88 production rates very low, ~1 barrel d<sup>-1</sup>) (mseel.org). On certain dates, the 5H well was not  
89 producing fluid so no sample was collected. Samples were refrigerated at 4 °C and shipped on  
90 ice within two weeks of collection. One field blank was collected by taking MilliQ water in to  
91 the field and then processed in the same manner as samples.

### 92 *2.2 Organics extraction*

93 Upon receipt, samples were filtered over a 0.7 µm glass fiber filter (Whatman GF/F) in to  
94 glass bottles previously baked at 500 °C. Filtered samples (200 mL) for solid phase extraction

95 were immediately acidified to pH 2 with concentrated hydrochloric acid and extracted over  
96 1g/6mL Bond Elut PPL solid phase extraction cartridges (preconditioned with 2 cartridge  
97 volumes of methanol followed by 2 volumes of 0.1% formic acid solution) (Dittmar et al., 2008).  
98 Loaded cartridges were desalted using a 200 mL dilute hydrochloric acid rinse (pH = 2) followed  
99 by a 30 mL 0.1% formic acid solution rinse to avoid halide contamination of the methanolic  
100 extract. Large volume washing of cartridges reduces the likelihood of iodo-adducts (Luek et al.,  
101 2017; Xu et al., 2013) and prior investigations with high and ultrahigh resolution electrospray  
102 mass spectrometry have confirmed covalently-bound iodine in complex mixtures (Luek et al.,  
103 2017; Moulin et al., 2001; Xu et al., 2013). Cartridges were dried under vacuum and eluted with  
104 10 mL ultrapure methanol. Methanolic extracts were stored at -20 °C prior to FT-ICR-MS  
105 analysis.

### 106 *2.3 FT-ICR-MS analysis*

107 Methanolic extracts were diluted 1:5 in ultrapure methanol and injected at 120  $\mu\text{L hr}^{-1}$   
108 using a Bruker Solarix 12T electrospray ionization FT-ICR-MS located at the Helmholtz  
109 Zentrum Munich, Germany. The instrument was operated in negative mode to target solid phase  
110 extracted compounds and target HOCs. Complementary positive ionization was not performed  
111 although this mode could have ionized additional organic compounds, including nitrogen  
112 containing HOCs. 500 scans were averaged for each sample and a post calibration was  
113 performed using a list of known DOM internal calibrants to obtain a mass accuracy of less than  
114 0.1 ppm (**Table S1**). The obtained full scan mass resolution was better than 400,000 at  $m/z$  400,  
115 allowing for precise formula assignments (Hertkorn et al., 2008). All  $m/z$  ions identified in the  
116 field blank were removed prior to further processing. Following the methods of Sleighter et al.,  
117 (2012) replicate sample mass spectra were compared to confirm that variability in the mass

118 spectral analysis across samples was different from variability among extraction replicates as a  
119 function of the % of overlapping  $m/z$  ions and a regression of peak magnitudes from two  
120 replicates (**Figure S2, S3**).

121 Because the ions of interest were not understood *a priori*, no surrogate or internal  
122 standards were added to samples prior to extraction or analysis, and hence why FT-ICR-MS is  
123 used as a semi-quantitative approach. Ion suppression issues caused by changes in the matrix  
124 were limited by diluting samples sufficiently as determined by the transient spectra. However,  
125 remaining extraction and ionization efficiency issues are not addressed using this non-targeted  
126 approach. For this reason, changes that would only be consistent with the expected changes in  
127 the matrix itself (i.e., consistently present in only unbroken fracturing fluids but absent in all  
128 flowback and produced waters, the largest contrast in the fluid matrices) were not discussed to  
129 limit false pattern identification.

#### 130 *2.4 Hierarchical Cluster Analysis*

131 Hierarchical cluster analysis was performed using Gene Cluster 3.0 and TreeView on log  
132 transformed ion abundances to limit clustering driven only by high intensity ions. Clustering  
133 analysis using average linkages was performed on uncentered  $m/z$  ions identified in each well on  
134 ions present in 2 or more samples between  $m/z$  150 – 400 (3H, n= 6613; 5H, n=5296). Clusters  
135 were selected with ions unique to flowback and early produced waters (first three months of well  
136 operation) and assigned formulas.

#### 137 *2.5 Formula Assignments*

138 Formulas were assigned to individual ions from the entire spectra including selected  
139 hierarchical clusters using in-house software (Hertkorn et al., 2008). A range of 150 - 700  $m/z$   
140 was selected to encompass the majority of ions in the spectra and where the calibration is reliable



141 to 0.1 ppm. Formulas were assigned with a maximum value per assignment of  
142  $C_{100}H_{\infty}O_{80}N_3S_2Cl_3Br_3I_3$  and a maximum error of 0.2 ppm. The mass error associated with ions  
143 identified below the lowest molecular weight calibrant was sufficient for formula assignment in  
144 this 0.2 ppm window. Nonsensical formula assignments were removed using a number of criteria  
145 in favor of alternative plausible formula assignments. Formulas not passing the nitrogen rule  
146 (McLafferty and Turecek, 1993) were removed within this software and remaining assignments  
147 were further reduced to remove invalid formulas by removing those with an oxygen to carbon  
148 ratio (O/C) greater than one or a negative double bond equivalent (DBE). Raw values of  
149 formulas containing only carbon, hydrogen, and oxygen (CHO), as well as nitrogen (CHON) or  
150 sulfur (CHOS) number between  $m/z$  150-700 are reported in **Figure S4**.

151 Additional filtering of assigned formulas identified during the cluster analysis involved  
152 removing assignments with more than 3 heteroatoms (e.g.,  $CHOI_3$  kept,  $CHOI_3S_1$  removed) and  
153 preferentially removing duplicate assignments with very low O/C ratios and higher heteroatoms  
154 based on consistencies found when checking many assigned formulas against isotopic pattern  
155 matching and consistently confirming their alternatives. For example, of the observed  $m/z$  ions  
156 assigned to an iodinated formula, approximately 50% had a duplicate formula assignment  
157 containing S and Cl, but were determined false assignments because the distinctive  $^{35}Cl$  to  $^{37}Cl$   
158 isotopic ratios were not observed in the mass spectra. It is possible that this stringent filtering  
159 criteria may have removed a small number of correct formula assignments but was necessary for  
160 management of the large dataset.

161 Halogenated formula assignments were compared to their theoretical isotopic patterns  
162 (**Figure S5**) and those ions not matching their theoretical isotopic distribution were removed  
163 (<0.1 ppm error with error consistent across isotopes and maximum 10% error in magnitude).

164 HOCs found using the cluster analysis with insufficient intensity to confirm isotopically were not  
165 removed (43% of HOC formulas). However, about half of these low intensity ions were  
166 members of a homologous series for which at least one member was confirmed isotopically.  
167 Degree of confidence for halogenated formula assignments is discussed in Section 3.1 and  
168 reported in **Table S4**. Assigned formulas matching known compounds are putatively named as  
169 such based on their plausible presence in these fluids and likelihood to ionize under the methods  
170 used, but have not been confirmed structurally.

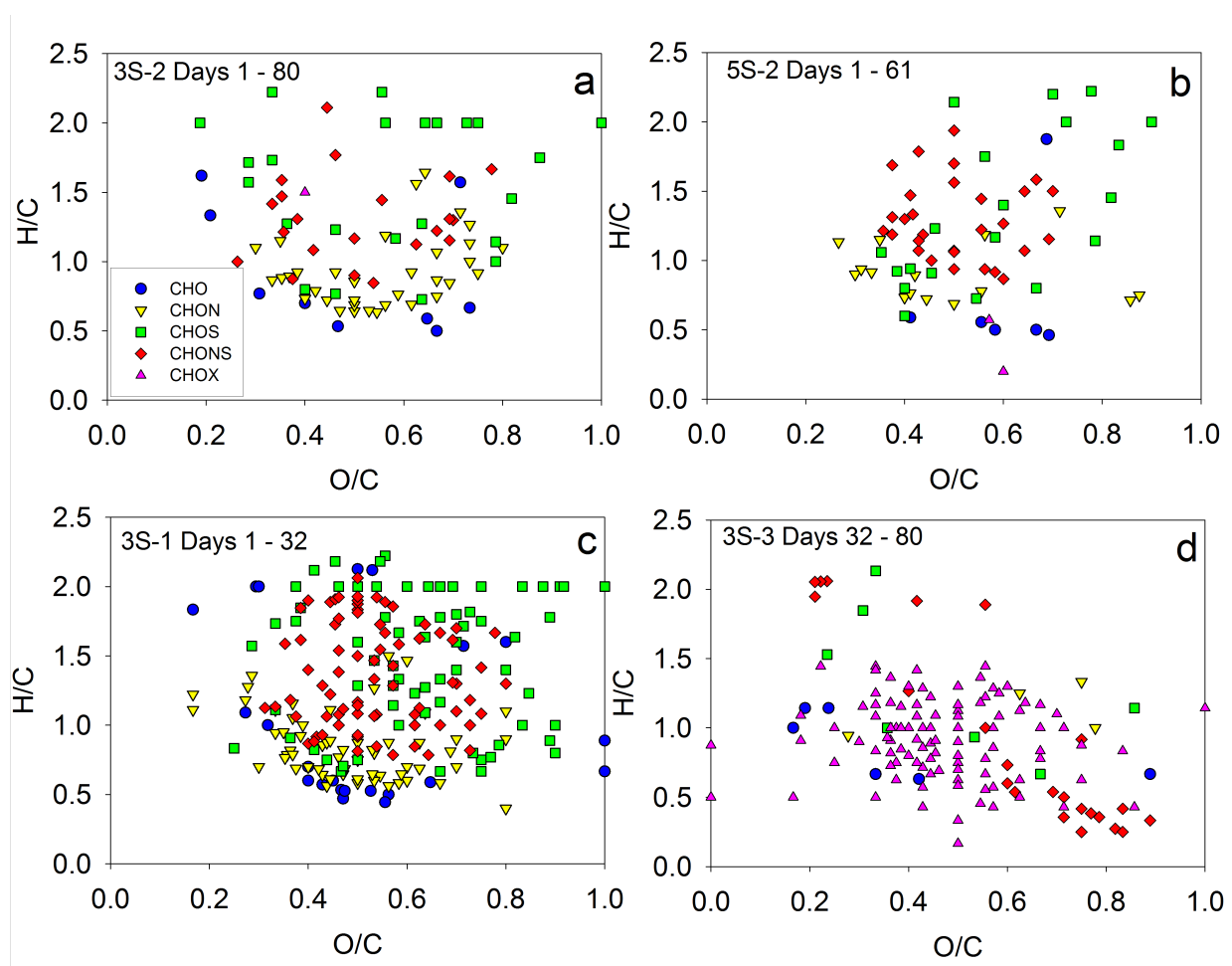
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## 172 **3 Results and Discussion**

### 173 *3.1 Cluster analysis reveals halogenated ions unique to early produced waters*

174 Due to the obvious differences in the injected fluid (unbroken gel) and the flowback and  
175 produced waters (broken gels & shale derived fluids), hierarchical clusters that only described  
176 differences between identified ions the injected fluid and flowback and produced waters were not  
177 compared to avoid improper comparisons based on possible extraction differences between these  
178 two fluid types. Instead, ions unique to a certain period of flowback were selected, absent both  
179 before and after the selected period and therefore both the broken and unbroken fluid types.  
180 Three clusters could be identified in the 3H well series that contained ions unique to different  
181 flowback and early produced waters, representing three fluid age groups (3S-1, 3S-2, and 3S-3)  
182 (**Figure S6**). The 5H well series contained fewer samples and only one cluster of ions unique to  
183 flowback and early produced waters could be identified (5S-2) (**Figure S7**). All  $m/z$  ions  
184 identified in these clusters are given in supplemental **Table S2** regardless of whether or not they  
185 could be assigned an unambiguous molecular formula.

186 The molecular formulas identified in the four selected clusters are presented as Van  
 187 Krevelen diagrams to visualize differences in the formula assignments as a function of  
 188 heteroatom type (Figure 1) (van Krevelen, 1950) and oxidation and saturation status. The  
 189 selected clusters contained a large number of heteroatom formula assignments rather than those  
 190 containing only carbon, hydrogen, and oxygen typically dominant in natural organic matter  
 191 (Table S3). The ions in clusters 3S-1, 3S-2, and 5S-2 contain similar DOM heteroatom classes  
 192 and distributions, with 74 ions shared between the 5H well cluster and either the 3S-1 or 3S-2  
 193 cluster. In contrast, only three of the 5H well cluster ions overlapped with those in cluster 3S-3.  
 194 A large number of ions containing both nitrogen and sulfur were observed.



195  
 196 **Figure 1.** Oxygen to carbon (O/C) ratio versus hydrogen to carbon (H/C) ratio of formulas  
 197 assigned to molecular ions (van Krevelen, 1950) for hierarchical clusters unique to flowback and

198 early produced waters a) 3S-2 b) 5S-2 c) 3S-1 d) 3S-3. Fluid ages for most ions in each cluster  
199 are given.

200  
201 Cluster 3S-3, representing ions present only in early produced waters (32-80 d), was  
202 distinct from the other clusters, containing 155 ions with halogenated formula assignments that  
203 could be validated to varying degrees (**Table S4**). The majority of ions were iodinated (52%),  
204 while 20% were brominated, 9% were chlorinated, and 19% contained two different halogens.  
205 Sixty-five of these halogenated formula assignments were supported with secondary peaks  
206 matching their theoretical stable isotopic spectra (e.g., **Figure S5**). Five iodinated ions had been  
207 previously identified in a North Dakota flowback fluid where their assignment was supported by  
208 the appearance of a 126.9045  $m/z$  peak (iodine) during fragmentation (Luek et al., 2017). Of the  
209 remaining ions assigned plausible halogenated formulas, many were members of homologous  
210 series [separated by  $\text{CH}_2$  groups determined using  $k_{m/z}$  values (Shakeri Yekta et al., 2012)]  
211 where at least one member of the series had been confirmed isotopically. Thirty-eight of the  
212 remaining halogenated formula assignments had intensities too low to rely on isotopic pattern  
213 matching (particularly iodinated assignments which rely solely on the  $^{13}\text{C}$  peak). Among the  
214 other three clusters, only five plausible halogenated formula assignments were identified and  
215 three confirmed using isotopic pattern matching.

216 The overlap of many nitrogen and/or sulfur containing compounds between the 3H well  
217 and the 5H well clusters suggests similar processes are occurring in both wells resulting in these  
218 ions unique to flowback and early produced waters. This is expected due to their similar  
219 hydraulic fracturing fluids and underlying geology. In contrast, the absence of a 5H well cluster  
220 containing HOCs suggests a possible differences between the two wells, possibly related to the  
221 smaller quantity of ammonium persulfate breaker added to 5H well (see Section 3.3).

222

### 223 3.2 Iodinated organic ions high in flowback and produced water

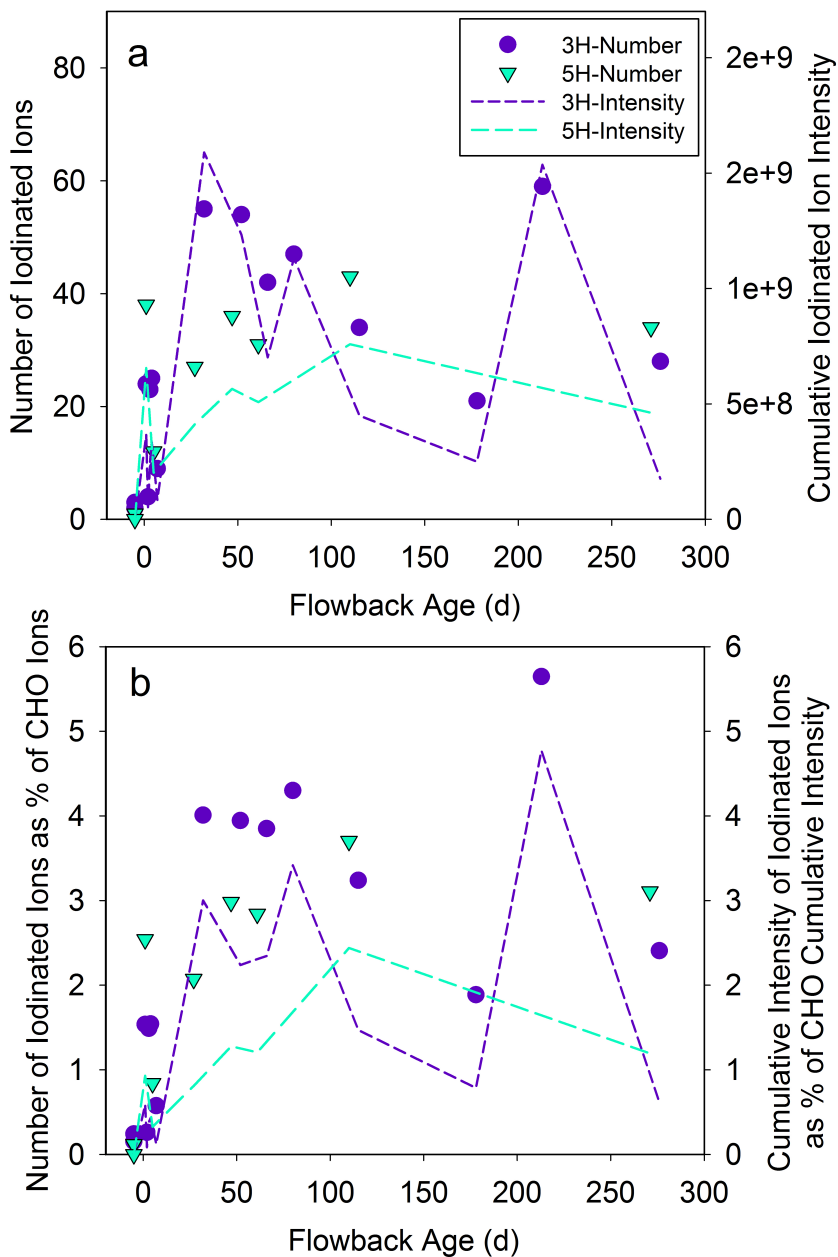
224 Due to the large number of iodinated organic compounds identified during the  
225 hierarchical cluster analysis, we further investigated the temporal dynamics of this specific class  
226 of compounds. Iodinated organic compounds are of particular interest due to their limited known  
227 natural occurrence (Dembitsky, 2006) and the toxicity of known iodinated disinfection by-  
228 products (Duirk et al., 2011; Plewa et al., 2004; Richardson et al., 2008). Iodinated organic  
229 compounds were tracked across all fluids in the time series to investigate this class of HOCs.  
230 Prior to injection, the fracturing fluids were nearly devoid of iodinated ions, with three or fewer  
231 identified at relatively low abundances in individual samples (**Figure 2a**). Beginning with the  
232 first week of flowback, the number of iodinated ions increased, and remained high in all  
233 produced water samples out to 276 days. The cumulative abundance of all iodinated ions also  
234 followed this trend, with higher intensities but more variability observed in the 3H well. A small  
235 volume (20 mL) quality control extraction was performed in August 2016 on all previously  
236 collected samples and showed the same trends over the time series. The small volume extractions  
237 had slightly lower numbers of ions and intensities, likely related to either the smaller sample  
238 volumes or the aging of the fluids prior to extraction (3-7 months storage unfiltered at 4 °C).  
239 Because of the expected variability in the make-up of the injected fluid and wastewaters over  
240 time, the changes in iodinated ion number and abundance were also computed relative to the  
241 number and cumulative abundance of CHO ions present in each fluid sample (**Figure 2b**). The  
242 same temporal trends are observed on the basis of raw number and abundance as are seen  
243 relative to CHO ions. The observed pattern is in contrast to the temporal trends for CHO, CHON,  
244 and CHOS ions over the time series (**Figure S4**). This supports that the observed trends are

245 indeed a function of actual changes in the fluid mixture rather than as a function of analytical  
246 differences due to ion suppression or extraction efficiency.

247         The majority of the iodinated ions confirmed with  $^{13}\text{C}$  isotopic pattern matching  
248 contained only carbon, hydrogen, oxygen, and one iodine atom, but seven ions contained  
249 nitrogen or sulfur (**Table 1**), and five ions contained two iodine atoms. The largest iodinated ion  
250 confirmed with  $^{13}\text{C}$  was  $m/z$  433.1206. Above this value, other peaks could be assigned iodinated  
251 formulas, but none had sufficient intensity to allow for confirmation with the  $^{13}\text{C}$  peak. All ions  
252 identified in the 5H well were also identified in the 3H well, but 11 iodinated ions present in the  
253 3H well were not identified in the 5H well. FT-ICR-MS provides no structural information, but  
254 some structures can be inferred based on the limited number of structural isomers for small  
255 compounds and their ability to be extracted and ionize under the experimental conditions. For  
256 example, the corresponding neutral formula  $\text{C}_2\text{H}_2\text{O}_2\text{I}_2$  is expected to be diiodoacetic acid, a  
257 known disinfection by-product (DBP) (Plewa et al., 2004) and naturally occurring compound  
258 (Dembitsky, 2006). However, most identified ions are large and therefore cannot be structurally  
259 determined without additional analyses.

260         The observed distribution of O/C and H/C ratios of these iodinated compounds (**Figure**  
261 **S8**) was consistent with the distribution of aromatic, particularly oxygen-rich and phenolic  
262 compounds (e.g., fulvic acids) susceptible to reaction with reactive iodine (Moulin et al., 2001).  
263 Halogenation of unsaturated compounds, aromatics, substituted aromatics (i.e., phenols) and  
264 natural organic matter can occur rapidly via electrophilic aromatic substitution (Criquet et al.,  
265 2015; Westerhoff et al., 2004). A similar distribution (based on O/C vs. H/C) of iodinated DBPs  
266 was formed during the chloramination of drinking water containing iodide, indicating the

267 preferential formation of iodinated compounds from these aromatic DOM precursors (Wang et  
 268 al., 2016).  
 269



270  
 271  
 272 **Figure 2.** MSEEL 3H and 5H iodinated ion number and cumulative iodinated ion abundance as  
 273 raw values (a) and as a percent of CHO number and cumulative abundance (b). Injected fluids  
 274 are shown as a time point prior to Day 1 of flowback.  
 275

276 **Table 1.** Iodinated molecular formulas supported with <sup>13</sup>C peak identified in 2 or more samples.  
 277 <sup>P</sup>Previously identified in fracturing fluid (Luek et al., 2017). <sup>S</sup>Exact masses also identified in  
 278 stream water DOM unrelated to hydraulic fracturing. <sup>3</sup>Ions unique to the 3H well.  
 279

Measured Mass	No. Times identified	H	C	O	N	S	I
236.9054 <sup>P</sup>	16	2	5	3	0	0	1
276.9367 <sup>S</sup>	16	6	8	3	0	0	1
282.9473	11	8	7	4	0	0	1
292.8952	8	2	7	5	0	0	1
292.9316 <sup>P</sup>	18	6	8	4	0	0	1
305.9633	3 <sup>3</sup>	9	9	3	1	0	1
306.9109	18	4	8	5	0	0	1
306.9473 <sup>S</sup>	17	8	9	4	0	0	1
308.9266	21	6	8	5	0	0	1
310.8072 <sup>P</sup>	7	1	2	2	0	0	2
320.963	17	10	10	4	0	0	1
322.8072	3 <sup>3</sup>	1	3	2	0	0	2
322.9422	19	8	9	5	0	0	1
322.9786	14	12	10	4	0	0	1
327.0099 <sup>S</sup>	21	16	10	4	0	0	1
334.9422 <sup>S</sup>	18	8	10	5	0	0	1
334.9786	13	12	11	4	0	0	1
336.9579	19	10	10	5	0	0	1
338.9194	9	8	9	4	0	1	1
338.9735	15	12	10	5	0	0	1
339.0099	2	16	11	4	0	0	1
342.9143	8	8	8	5	0	1	1
343.0048	14	16	10	5	0	0	1
345.0205	14	18	10	5	0	0	1
350.9372	19	8	10	6	0	0	1
350.9735	16	12	11	5	0	0	1
351.0099	2 <sup>3</sup>	16	12	4	0	0	1
352.9528	16	10	10	6	0	0	1
352.9892	18	14	11	5	0	0	1
355.0048 <sup>P</sup>	12	16	11	5	0	0	1
355.0412	2 <sup>3</sup>	20	12	4	0	0	1
357.0205	4	18	11	5	0	0	1
364.9528 <sup>S</sup>	17	10	11	6	0	0	1
367.0049	7	16	12	5	0	0	1
369.0205	5 <sup>3</sup>	18	12	5	0	0	1
370.0521	2	21	12	4	1	0	1



376.9528 <sup>S</sup>	17	10	12	6	0	0	1
376.9891	11	14	13	5	0	0	1
378.9684 <sup>S</sup>	18	12	12	6	0	0	1
379.0048	9	16	13	5	0	0	1
381.0205	10	18	13	5	0	0	1
383.0361	3 <sup>3</sup>	20	13	5	0	0	1
387.031	4 <sup>3</sup>	20	12	6	0	0	1
392.9476 <sup>S</sup>	18	10	12	7	0	0	1
397.0154	11	18	13	6	0	0	1
399.0311 <sup>S</sup>	7	20	13	6	0	0	1
401.0467	2 <sup>3</sup>	22	13	6	0	0	1
402.8334	5	5	8	3	0	0	2
404.00	3 <sup>3</sup>	15	14	5	1	0	1
406.9633 <sup>S</sup>	16	12	13	7	0	0	1
420.8076	5	3	7	5	0	0	2
421.0153	9	18	15	6	0	0	1
423.031	10	20	15	6	0	0	1
425.0467	11	22	15	6	0	0	1
427.026	8	20	14	7	0	0	1
427.0623	6	24	15	6	0	0	1
428.7796	9	3	5	5	0	1	2
433.1206	20	30	14	5	2	0	1

280

281 *3.3 Possible origins of halogenated organic compounds*

282 Four sources have been proposed for the origin of HOCs in flowback fluids: a) chemical  
 283 additives in the hydraulic fracturing fluid b) leached shale molecules c) biotic reactions between  
 284 additives and/or shale compounds and d) abiotic reactions between additives and/or shale  
 285 compounds (Hoelzer et al., 2016; Luek et al., 2017; Maguire-Boyle and Barron, 2014). The  
 286 specific additives reported for MSEEL wells on the FracFocus database (fracfocus.org) do not  
 287 contain HOCs, so this is an unlikely source.

288 Thousands of naturally occurring HOCs do exist (Gribble, 2010), but the number of  
 289 known iodinated compounds is limited, with just over 110 compounds identified in the published  
 290 literature (Dembitsky, 2006). We searched all samples for the exact masses of all known  
 291 biogenic iodinated organic compounds (Dembitsky, 2006) and identified four. Three of these

292 four were supported with their  $^{13}\text{C}$  peak, diiodoacetic acid ( $m/z$  310.80717), iodotyrosine ( $m/z$   
293 305.96326) and iodophloroglucinol ( $m/z$  376.95276, also identified in fracturing fluid prior to  
294 injection), while diiodomethane ( $m/z$  266.81732) was present at low abundances so the expected  
295  $^{13}\text{C}$  peak would be below the baseline and therefore could not be confirmed. To this end, the  
296 Marcellus shale could contain HOCs derived from ancient biogenic processes that were not  
297 remineralized during diagenesis and slowly leached from the shale as the wells matured.  
298 However, many biogenic halogenated formulas, such as methyl halides and halogenated phenols  
299 can be microbially degraded (Gribble, 2010), and likely would not persist in the environment  
300 over geologic timescales. Although the HOC content of shales is poorly characterized, their  
301 probable degradation indicates that an ancient biogenic origin for the identified HOCs is  
302 unlikely.

303 In addition to their possible sourcing as natural biogenic products, diiodomethane and  
304 diiodoacetic acid can also be formed as disinfection by-products (DBPs). These two compounds  
305 were not the only putatively identified DBPs in this dataset; several other exact masses also  
306 matched known DBPs. The exact masses of DBPs detected only in flowback and produced  
307 waters included those matching halogenated acetic acids, iodomethylbutenedioic acid, and  
308 several halogenated aromatic structures (halogenated benzaldehydes, benzoic acids, phenols, and  
309 benzoquinones). Halogenated DBPs can be formed when oxidizing chemicals such as  
310 chloramines and persulfates react with halides to form reactive halogen species, which  
311 subsequently react with DOM and xenobiotic compounds (Gong and Zhang, 2015; Plewa et al.,  
312 2004; Postigo et al., 2016; Wang et al., 2016; Xie et al., 2015). Of the identified iodinated  
313 molecular formulas (**Table 1**), all but 7 had their non-iodinated counterparts (replacing I with H)  
314 present in MSEEL samples and were also present at high intensity ( $>10^8$ ).

315 Ammonium persulfate (listed as diammonium peroxodisulphate on the FracFocus report),  
316 a strong oxidizing agent, was used in both the 3H and 5H well hydraulic fracturing fluids  
317 (fracfocus.org). Persulfate oxidation has been used as an advanced oxidation process in  
318 wastewater treatment, relying on the activation of persulfate (via heat, UV light, ultrasound, or  
319 an electron) to form two sulfate radicals (Matzek and Carter, 2016). High temperatures found in  
320 the Marcellus Shale at depth and many potential electron donors (e.g., transition metals,  
321 additives) could activate the added persulfate. The resulting sulfate radicals are highly reactive,  
322 and can propagate a number of diverse reactions beyond their intended role of breaking  
323 polymers. Persulfate oxidation can form reactive halogens including iodine, hypiodite, radical  
324 iodine, and others that can ultimately react with organic compounds including DOM and phenols  
325 to form DBPs (Lu et al., 2015; Wang et al., 2017). The observation of more diverse HOCs  
326 present in the 3H well than the 5H well is consistent with a persulfate source for these  
327 compounds, as ammonium persulfate was applied at a concentration 75 times higher in the 3H  
328 well than the 5H well (0.00074% vs. 0.00001% by mass of hydraulic fracturing fluid).  
329 Additionally, reactive iodine species involved in these halogenation reactions can also be formed  
330 through natural processes (without the external addition of oxidant additives) through abiotic  
331 reactions with DOM (Li et al., 2012) and oxidation of iodide to reactive iodine by biotically  
332 produced hydrogen peroxide and organic acids (Steinberg et al., 2008).

333 Bacteria known to oxidize iodide have been identified in hydraulic fracturing  
334 wastewaters previously (Amachi et al., 2005; Murali Mohan et al., 2013), and may be an  
335 additional source of reactive iodine. These bacteria are capable of converting inorganic iodide to  
336 reactive iodine that can subsequently react with DOM along the same pathways as abiotically-  
337 produced reactive iodine. Although biocides are employed to limit bacterial growth, diverse and

338 active communities are found in these fluids downhole (Cluff et al., 2014; Daly et al., 2016;  
339 Mouser et al., 2016; Murali Mohan et al., 2013), with hydraulic fracturing increasing the shale  
340 poresize and removing this physical limitation for microbial life in the deep shales (Mouser et  
341 al., 2016). We searched for taxa phylogenetically associated to known iodide-oxidizing bacteria  
342 in 16S rRNA data from MSEEL samples. Taxa closely related to uncultured *Roseovarius spp.*  
343 were observed in MSEEL 3H drilling muds (2-4%) as well as flowback and early produced  
344 fluids (from 2 through 119 days) from both the 3H and 5H wells (<1%) (**Figure S9, Table S5**)  
345 (unpublished data from Kelly Wrighton, for methods see Cluff et al., 2014; Daly et al., 2016).  
346 Some *Roseovarius spp.* are capable of iodide oxidation in conjunction with production of  
347 iodinated organic compounds, including methyl halides (Amachi et al., 2005; Fuse et al., 2003).  
348 This reaction requires iodide, an oxidant (e.g., peroxide), and appropriate genes (i.e. halide  
349 peroxidases) that are poorly characterized in bacteria. As mineral iodides and oxidants (e.g.  
350 persulfate) are present in this system, these data suggest the potential exists for biotic production  
351 of iodinated organic compounds in conjunction with abiotic reactions in this system, albeit by  
352 low abundance microbial community members.

353

#### 354 **4. Conclusions**

355 Iodinated organic ions were tracked through the first nine months of operation of two  
356 Marcellus Shale gas wells using FT-ICR-MS and revealed a steep increase in the number of ions  
357 assigned iodinated organic molecular formulas during the initial flowback period. The number  
358 and abundance of iodinated organic ions remained elevated in produced waters 276 days later.  
359 Hierarchical clustering analysis also revealed a large number of iodinated, brominated and  
360 chlorinated ions that were unique to fluids returning to the surface of the 3H well 1-3 months

361 after the initial flowback. We suggest that biotic and abiotic oxidation of halides subsequently  
362 reacting with diverse DOM contributes to the observed organohalogen diversity and temporal  
363 dynamics. Tracking changes in the chemical composition of shale gas fluids is essential for  
364 understanding fundamental changes occurring in hydraulic fracturing fluids, particularly those  
365 driven by known additives and microbial communities. Although many similarities were  
366 observed between the two wells, their differences raise questions as to why these differences  
367 exist: Do they reflect differences in geology, hydraulic fracturing fluid mixtures, hydraulic  
368 fracturing techniques, or some other undescribed variable? More work on this topic is needed to  
369 better understand how these results can be generalized to different wells. Understanding why  
370 differences are observed across hydraulic fracturing wastewaters is essential for understanding  
371 the fundamental functions occurring within a well, and for addressing more applied questions of  
372 which wastewaters are suitable for reuse or a given treatment technique.

373

#### 374 **Supplemental Materials**

375       Supplementary data related to this article can be found at [http://](http://dx.doi.org/10.1016/j.watres.XXXX)  
376 [dx.doi.org/10.1016/j.watres.XXXX](http://dx.doi.org/10.1016/j.watres.XXXX). Additionally, calibrated mass lists for all samples with ions  
377 identified using FT-ICR-MS are available through the Dryad digital repository  
378 (<http://datadryad.org/>).

379

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