Organic Geochemistry and Toxicology of a Stream Impacted by Unconventional Oil and Gas Wastewater Disposal Operations

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Abstract

The large volume of wastewater produced during unconventional oil and gas (UOG) extraction is a significant challenge for the energy industry and of environmental concern, as the risks due to leaks, spills, and migration of these fluids into natural waters are unknown. UOG wastewater is often hypersaline, and contains myriad organic and inorganic substances added for production purposes and derived from the source rock or formation water. In this study, we examined the organic composition and toxicology of water and sediments in a stream adjacent to an underground injection disposal facility that handles UOG wastewaters. We sampled water and streambed sediments from an unnamed tributary of Wolf Creek upstream from the disposal facility, near the injection well, and downstream. Two sites downstream from the disposal facility contained organic compounds in both water and sediments that were consistent with a source from UOG wastewater. These compounds included: 2-(2-butoxyethoxy)-ethanol, tris(1-chloro-2-propyl)phosphate, α , α -dimethyl-benzenemethanol, 3-ethyl-4-methyl-1H-pyrrole-

2,5-dione, and tetrahydro-thiophene-1,1-dioxide in water, diesel fuel hydrocarbons (e.g. pentacosane, Z-14-nonacosane), and halogenated hydrocarbons (e.g., 1-iodo-octadecane, octatriacontyltrifluoroacetate, dotriacontylpentafluoropropionate) in sediments. Concentrations of UOG-derived organic compounds at these sites were generally low, typically 4 to <1 µg/L in the water, and <70 µg/g (dry wt.) in the sediment. In addition, water and sediment at a site immediately downstream from the facility contained many chromatographically unresolved and unidentified hydrocarbons. In contrast, sites upstream from the facility or in nearby watersheds not influenced by the disposal well facility contained primarily natural (biologically produced) organic substances from the local environment. Toxicological assays of human cell line exposures to water and sediment showed minimal effects. Results indicate that UOG wastewater has entered the stream and that UOG-derived organic substances are present. The contamination level, however, is low and appears to be restricted to sites immediately downstream from the disposal facility at this time.

1. Introduction

The disposition of large volumes of wastewater is one of the biggest issues facing unconventional oil and gas (UOG) production. UOG is a general term for oil and gas production that does not meet the criteria typically applied to conventional oil and gas production, and includes: shale gas, coalbed methane, tight gas, oil shale, oil sand, extra heavy oil, and gas hydrates (Ratner and Tiemann, 2015). UOG may employ horizontal drilling and hydraulic fracturing as production tools. The volume of UOG wastewater has strained the handling and disposal capacity in the USA (Lutz et al., 2013). Although significant progress has been made in recycling water used in hydraulic fracturing and reducing the amount of wastewater, much of it still needs to be disposed of, usually by deep underground injection as these wastewaters are unsuitable for disposal in standard wastewater treatment plants (Soeder and Kappell, 2009; Engle et al., 2014).

Water quality considerations are a critical issue in the disposition of wastewater from UOG production (EPA, 2012). Wastewater from UOG has a complex chemistry that includes chemicals added for

hydraulic fracturing and other production purposes (e.g. biocides, corrosion inhibitors, degreasers, etc.), substances from the formation water (high dissolved ion content-hypersalinity), and substances mobilized from the targeted formation (e.g. naturally occurring radioactive materials). Chemical characteristics of the wastewater of environmental concern include: organic compounds (Orem et al., 2014, Thurman et al., 2014; Hladik et al., 2014; Akob et al., 2015), toxic metals and metalloids (Lutz et al., 2013), radionuclides (Brown, 2014; Chapman et al., 2012; Rowan et al., 2011), and high salinity (Rowan, 2011; Haluszczak et al., 2013; Rowan et al. 2015). Some of the identified chemical substances have potential health impacts, and include carcinogens such as polycyclic aromatic hydrocarbons, neurotoxins such as isopropanol, irritants such as sodium persulfate, and reproductive and endocrine toxins such as ethylene glycol and nonylphenols (Kassotis et al., 2014 and 2015; Webb et al., 2014). Some of the organic substances present, such as glutaraldehyde, 2-butoxyethanol, 2,2-dibromo-3-nitrilopropionamide, and dibromoacetonitrile, may be toxic at low (ppb) concentrations (U.S. Department of Health and Human Services, 1993a and b). Environmental and human health impacts from UOG production may result if exposures occur from release of wastewater due to spills during UOG production activities, or improper handling, storage, transport, or disposal of produced water (Werner et al., 2015).

In this paper, we present results from a study of the organic geochemistry and human cell line toxicology of surface water and sediments from a small creek near a Class II underground injection control (UIC) well facility which disposes of UOG wastewaters. Results for inorganic parameters and impacts on the microbial community are reported in Akob et al. (2016), and for endocrine disrupting activities in Kassotis et al. (2016). This study is part of a broader effort by the USGS Toxic Substances Hydrology Program to investigate the environmental and human health impacts of UOG wastes (<http://toxics.usgs.gov/investigations/uog/>). It was hypothesized that organic compounds from UOG wastewater might be present in the surface water and sediment of the creek near the disposal facility, and that these compounds may represent a potential health risk. Targeted organic compounds were based on previous studies of organic substances present in UOG wastewater, and lists of compounds used in UOG

production (U.S. House of Representatives Committee on Energy and Commerce Minority, 2011; Orem et al., 2014; Hladik et al., 2014; Kelland, 2014; Maguire-Boyle and Barron, 2014). Potential pathways for wastewater from the disposal facility to enter surface water or groundwater may include: direct, accidental release from tanker trucks at the site, leakage from onsite storage ponds or tanks, migration of wastewater through subsurface aquifers at the injection depth or through failed casing in the UIC well, and runoff of contaminants spilled on the well pad in rainwater or snow melt.

2. Site Description

Water and stream bottom sediment samples were collected from Wolf Creek and nearby small tributaries, with work focused on an unnamed tributary adjacent to a UIC disposal facility located near Lochgelly, West Virginia (WV; Fig. 1). Wolf Creek discharges into the New River which is a drinking water supply for the city of Fayetteville, WV. The injection well was originally drilled in 1981 to a depth of 990.6 m as a coalbed methane (CBM) production well. The well was reworked as a Class II UIC well in 2002, with fluids injected into sandstone at a depth of 792.5 m. Different types of wastewater are accepted for injection at this site, including wastewater from UOG production in the Marcellus Shale and CBM (as described in Akob et al. 2016). As originally configured, wastewater transported to the site in tanker trucks was transferred to impoundment ponds (synthetic liner) on the site where solids in the water were removed by settling. After settling, wastewater was injected into the well. The settling pond operated from 2002 to 2014 when it was closed and filled in. The pond was still in operation during our sampling in 2013, but not during later sampling in 2014. Wastewater is now apparently directly injected underground, or stored in brine holding tanks at the site. Additional description of the site may be found in Akob et al. (2016).

All sites sampled are shown in Fig. 1, and descriptions of each site are listed in Table 1. Site 1 is not included here as it is not pertinent to the discussion. Sites 3-7 are located on an unnamed tributary of Wolf Creek near the disposal facility; Site 4 is slightly upstream from the facility; Site 5 is at the same level as

the well pad and upstream from the settling pond; Site 6 is two sites (6 and 6A), with 6 located adjacent to the injection well at a point along the drainage where it appears to pass under the (now filled in) settling pond (Akob et al., 2016), and 6A located where the drainage reemerges from the settling ponds. Results from Sites 6 and 6A are included in this report. Site 7 is downstream from Site 6 and directly across from the settling pond, and Site 3 is located about 200 m downstream from Site 7 where the stream merges with another tributary. Sites 9 and 10 are located along the main flowpath of Wolf Creek, but much further downstream (approximately 10 and 4 km, respectively). Sites 2, 11, and 12 are located on separate tributaries of Wolf Creek, with no potential impacts from the disposal facility or known oil and gas inputs. Site 8 is along Laurel Creek, a separate drainage from Wolf Creek that also discharges into the New River. The sites along other drainages provide background water quality information in this area that contains coal, old coal mines and waste associated with coal mining, possibly acid mine drainage, and other sources of contamination from domestic sources and light industry.

3. Methods

3.1 Sampling

Samples (surface water and sediment) were collected in August and September 2013, and June and September 2014. Water samples were analyzed for non-purgeable dissolved organic carbon (NPDOC) and extractable hydrocarbons on all sampling dates, and for C₁-C₆ hydrocarbons, targeted UOG biocides, and organic disinfection byproducts (DBPs) in June 2014. Sediments were analyzed for total carbon (TC), total nitrogen (TN), total sulfur (TS), and extractable hydrocarbons only.

Water samples for extractable hydrocarbons and NPDOC were collected directly from the approximate center of the stream by submerging pre-cleaned bottles (1 L amber glass) to collect samples without headspace. Samples were stored in coolers on ice until filtration. Subsamples for NPDOC were filtered using Whatman® Polyethersulfone (PES) 0.2 µm syringe filters into pre-cleaned 60 mL amber glass vials. Filtered NPDOC samples were kept cool on ice in the field and later refrigerated in the lab until

analysis. Subsamples for analysis of extractable hydrocarbons were vacuum filtered through Whatman® GF/F 0.7 µm filters using an all glass Millipore filtration apparatus. The filtrate was transferred into pre-cleaned 1 L amber glass bottles with Teflon lined screw caps (filled to the shoulder), and 10 mL dichloromethane (DCM) was added to the filtrate in the field to prohibit biodegradation. Amber bottles were used to limit photodegradation of NPDOC and extractable hydrocarbons. Glassware and glass bottles were pre-cleaned either by baking in a furnace (450° C for 2 hours) or by washing with DCM (Orem et al., 2007).

Samples for low level C₁-C₆ dissolved hydrocarbon analysis were collected without filtration or headspace in 1 L amber glass bottles using polycone seal caps, and preserved with KOH. Water samples for analysis of DBP were collected without filtration or headspace in 1 L amber glass bottles, tightly capped, and stored on ice or refrigerated until analysis. Water samples for biocides were collected using a peristaltic pump (Geopump™ Peristaltic Pump Series II, Geotech Environmental Equipment, Inc. Denver, CO) through an in-line filter (Nuclepore 0.2 µm) into clean 1 L amber glass bottles. Filtered samples for biocides were stored chilled on ice or refrigerated until analysis.

Field blanks, consisting of reagent (ASTM Type I) water taken into the field in clean glass bottles and processed in the same manner as stream water samples, were used to assess any contamination from sample handling or processing. Field blanks consistently showed little or no contamination. Blank values for DOC reflected the DOC content of pure reagent water (<0.01 mg/L), and extractable hydrocarbon blanks consisted of little other than small peaks for phthalates (<1 µg/L), possibly from the plastic coating on the amber glass bottles.

Sediment samples were collected from the upper 1 - 2 cm of the soil surface or creek bottom using a clean stainless steel scoop. Fine-grained sediments were targeted where present, and visible twigs, leaves, and pebbles were removed from the sample with clean forceps. Sediment samples, as well as surface

vegetation that was collected for toxicology analysis, were stored in zip-lock plastic bags (double-bagged) and retained on ice or in a freezer until analyses.

3.2 Analytical Methods

NPDOC was determined using a Shimadzu TOC-VCPH analyzer equipped with a catalytically-aided 680 °C combustion chamber and normal sensitivity catalyst. Standardization was based on a 5-point calibration curve using an aqueous potassium phthalate stock standard (100 or 10 mg/L, depending on the DOC range of the samples); dilutions were performed in the instrument. The detection limit was approximately 0.3 mg/L based on laboratory determination. ASTM Type I Reagent Water was used as a blank, and each sample was analyzed at least four times to ensure data repeatability.

Sediments were analyzed for TC, TN, and TS using LecoTruSpec[®] C,H,N and Sulfur Analyzers (Leco Corporation, St. Joseph, Michigan). Wet sediment was lyophilized to remove all water, ground to homogenize using a mortar and pestle, and stored in glass vials; we did not sieve samples to remove sand. Prior to analysis, sediments were dried overnight at 60°C to remove adsorbed water, and cooled to room temperature in a desiccator. For TC and TN, samples ranging from 25 to 50 mg are weighed into tin sampling cups and placed in an autosampler for analysis. For TS, samples ranging from 1 to 10 g were weighed into ceramic combustion boats, covered with a vanadium pentoxide or tungsten oxide accelerant, and transferred into an autosampler for analysis. Samples were run at least in triplicate to check for repeatability?. Reference standard chemicals (e.g. EDTA, sulfamethazine) are used for calibration and Leco or National Institute of Standards and Technology (NIST) standard reference materials (e.g. soil, coal, ore tailings) used to check for accuracy during analysis. Blanks consisted of empty tin cups for TC and TN, and ceramic boats containing accelerant for TS. Results are reported as percent of sediment mass on a dry weight basis.

C₁-C₆ hydrocarbons were measured in water using purge-and-trap gas chromatography (GC) with a combination of an Atomic Emission Detector (AED) and a Flame Ionization Detector (FID) connected to

separate columns using a split-loop. This is an extension of the method in Haase et al. (2014), and includes the addition of a second sample loop that injects 0.2 mL of sparged gas into 2 m, 1/8" (3.1mm) OD, 80/100 mesh Heysep-Q column (VICI) terminating in a FID for measuring methane and high concentrations of compounds that otherwise would overwhelm the AED and be unquantified. The composite GC-AED/FID system is capable of quantifying dissolved light hydrocarbons over a concentration range of 10^{-14} to 10^{-3} mole/kg (10^{-10} to 10 mg/L) with >10% relative standard deviation (RSD) precision. In this study methane, ethane, propane, *n*-butane, *n*-pentane, and *n*-hexane were quantified against NIST traceable gas standards ranging from µg/L to mg/L levels (Scotty/Air Liquide).

Details of the method used for analysis of extractable hydrocarbons in water are found in Orem et al., 2007. In brief, extractable hydrocarbons in water were isolated by liquid/liquid extraction with four sequential volumes of 60 mL each of pesticide-grade dichloromethane (DCM); (Sigma-Aldrich, St. Louis, MO, USA). The combined DCM extract was concentrated by rotary evaporation and by evaporation under a gentle stream of N₂ in a nitrovap (Parker Balston, Haverhill, Massachusetts, USA) to 200 µL. A sub fraction (generally 2 µL) was used for analysis by gas chromatography/mass spectrometry (GC/MS). ASTM Type I Reagent Water was extracted for laboratory blank runs. Organic compounds were identified by comparison of mass spectral features to libraries of mass spectral data, and by comparison to standard compounds when available. Many peaks in the GC/MS chromatograms were not identified by the mass spectral libraries with sufficient accuracy and are not reported. Quantification of identified organic compounds was accomplished using authentic standards where available. If appropriate standards were not available (typically the case in this study), a standard mixture of target polycyclic aromatic hydrocarbons (PAHs) was used instead (see supplemental material for details). Because many compounds were not quantified with a standard for each specific compound, these results should be considered as semi-quantitative estimates.

Sediment samples were microwave extracted with a solvent mixture to isolate extractable hydrocarbons for analysis. Microwave extraction (Digestion System Accelerated Reaction Microwave, CEM Corp.,

Matthews, North Carolina, USA) was accomplished by adding 5.0 g of wet sediment sample and 5.0 g of anhydrous sodium sulfate to an extraction tube with 40 mL of a 50/50 (v/v) acetone/DCM solution. The temperature was ramped to 115 °C over 8 minutes and then held for 15 minutes. The pressure in the extraction tube at 115 °C is approximately 100 psi. Solutions were cooled and then filtered through clean (baked at 450 °C for 2 hours) Whatman (GE Healthcare Life Sciences, Pittsburgh, Pennsylvania, USA) GF/F filters (0.7 µm pore size). The filtrate was concentrated by rotary evaporation and then further reduced under a gentle stream of nitrogen to 1.0 mL and stored in a clean glass vial in a freezer until analysis. A sub fraction (generally 2 µL) was used for GC/MS analysis. Sand that was baked at 450 °C was used for laboratory blank runs. Details of the GC/MS analytical methodology may be found in the Supplemental Material.

A suite of biocides used in UOG production were screened in water samples from Wolf Creek on an Agilent Bioinert 1200 SL+ liquid chromatograph / 6460 triple quadrupole mass spectrometer with a Jet Stream electrospray ionization source in multiple reaction monitoring mode. Compounds included: (1) 3-hydroxy-5-methylisoxazole, (2) 2-methylthiobenzothiazole, propiconazole, (3) 2,2-dibromo-2-nitrilopropionamide (DBNPA), (4) 2-bromo-2-nitropropane-1,3-diol (Bronopol), (5) 4,4-dimethyl-oxazolidine, (6) Chlorhexidine, and (7) diiodomethyl-*p*-tolylsulfone. Compounds were gradient separated using a 0.1% formic acid (aqueous) versus 0.1% formic acid (methanol) on a Waters Atlantis T3 analytical column (3.0 x 100 mm). Only biocides (1), (2), and (3) were able to be quantified at this time, while the others were identified but concentrations could only be qualitatively determined. Analytical methods are still in development for these and other biocides used in UOG production.

Water samples for DBPs were filtered through baked (450 °C for 2 hours) 0.7 µm GF/F filter and then extracted using an Oasis HLB solid-phase extraction (SPE) cartridge (6 cm³, 500 mg; Waters Corporation, Milford, MA). DBPs were eluted from the cartridge with 10 mL of methyl tertiary butyl ether (MTBE). The MTBE was evaporated to 400 µL and after an internal standard (1-chlorooctane) was added, the samples were placed in vials with no headspace. Extracts were analyzed for 29 DBPs

(including trihalomethanes, haloacetonitriles and halonitromethanes) on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer (Santa Clara, CA). Specific conditions used for the DBP analysis are found elsewhere; method detection limits ranged from 0.02 to 0.2 µg/L (Hladik et al., 2014). Quality control for the DBP samples included a field blank, matrix spike and replicates (6); additionally every sample was spiked with surrogate compounds (1-bromo-4-fluorobenzene and d₄-1,2-dichlorobenzene). No DBPs were detected in the blank; matrix spike and surrogate recoveries ranged from 75 to 108%; no DBPs were detected in any of the samples or their respective replicates.

3.3 Human Cell Line Toxicology

A general description of the toxicology approach and assays used is presented here; for more details on the methods used the reader is directed to the Supplemental Material. We used human liver and kidney cell lines in the toxicology studies reported here. The cell lines were exposed to unfiltered water samples, water extracts of sediments, or water extracts of plant material from many of the same sites sampled for water chemistry in this report and elsewhere (Kassotis et al., 2016; Akob et al., 2016). These cell lines represent the two major systems (liver and kidney) in the body that are used for metabolism of toxic substances (conversion into metabolic intermediates, followed by elimination). Acute toxicity (24 hrTox) and clonal efficiency (CE) screenings were carried out using a transformed human liver cell line (HepG2 hepatocellular carcinoma; ATCC, #77400, Manassas, VA) which is a standard cell line for preliminary toxicity screening. We chose to use HK-2 kidney cells (HK-2, CRL 2190, ATCC, Manassas, VA) for the anchorage independent growth (AIG) assay (Ke et al., 2004) because of the unique metabolism, filtration, and concentration functions of the kidney, and because the kidney is uniquely susceptible to damage from exposure to xenobiotics. Specifically, the HK-2 cell line is a non-tumorigenic cell line that has been immortalized using recombinant human HPV-16 virus sequences, and grows as an adherent single-cell layer. The cell line has normal cellular metabolism, growth, adhesion, attachment and spreading characteristics. HK-2 reproduces results obtained from freshly-isolated proximal tubule cells. The HK-2 cell line will not grow anchorage-independently. Therefore in the AIG assay, cells must undergo an

adaptive mutation in order to survive and proliferate. The detailed protocols used for the 24 hr Toxicity Testing, and AIG and CE assays are presented in the Supplemental Material. For all *in vitro* assays, stocks were within 5 passages of each other for each of the replicate assays, and stocks >15 passages from initial passage number at receipt from ATCC were not used. If cells appeared to have changed significantly in growth or morphology, they were discarded in favor of earlier passage number vials.

The results from three assays each were averaged and means and standard deviations were calculated. All assays were done in triplicate (N=3) on at least two separate occasions. Results that differed by $p < 0.05$ were deemed statistically significant. Error bars for the graphical presentation of results represent the standard error of the mean.

4. Results and Discussion

4.1 NPDOC and Sedimentary Carbon

NPDOC content in water at all sites ranged from 1.13 to 7.35 mg/L (Table 1). The highest concentration was recorded at Site 7 in September 2013 and the lowest concentration was recorded at Site 4 in June of 2014. Overall, Sites 2 and 4 had the lowest NPDOC concentrations. Site 4 is upstream from the UIC well, and Site 2 is in a separate drainage. Sites 3 and 7 had higher DOC concentrations compared to Site 4 (along the same drainage but upstream of the UIC well) and Site 2 (background creek). This suggests that DOC from the disposal facility is entering the creek. This could be organic matter on the well pad washed into the stream during rain events, overflow or leakage of DOC from the settling pond, or other sources. Several sites further downstream from the injection well (Sites 9 and 10) also had slightly higher DOC values compared to Sites 2 and 4.

Sediments in these drainages had TC values ranging from 0.34 to 3.13% (dry wt.; Table 1). It was hypothesized that activities at the disposal facility might result in higher TC values in sediments immediately downstream (e.g., Sites 3 and 7); however, this was not observed. Sedimentary TN and TS values ranged from 0.12 to 0.22% and 0.02 to 0.07%, respectively (Table 1). TN and TS content tended to

follow TC, in general. Elevated TN and TS values were not observed at Sites 3 and 7 directly downstream from the injection well and settling pond. Thus, sedimentary TC, TN, and TS appeared to be controlled primarily by natural sources in the area (e.g. vegetation, algae).

4.2 C₁–C₆ (Light) Hydrocarbons in Surface Water

Although gas phase light hydrocarbons are effectively removed from the produced water at the separator and as condensate, some C₁–C₆ hydrocarbons may remain dissolved in the wastewater from UOG production. It was originally hypothesized that C₁–C₆ hydrocarbons from UOG wastewater might be present in surface water from sites near the UIC well. However, dissolved light (C₁–C₆) hydrocarbons concentrations were generally low in stream water (Fig. 2), and in the range expected from equilibrium with ambient air (Brasseur et al., 1999). The substantial variation observed in replicate samples usually indicates that the volume of the collected sample is of the same order as the stream flow rate in small drainages like this. Thus, the act of sampling may have perturbed the concentrations in the water at the sampling site by drawing water downstream at an accelerated rate and causing mixing. Even with the observed variation, however, the samples collected immediately downstream from the disposal facility (Sites 3 and 7) have higher overall concentrations of C₁–C₆ dissolved light hydrocarbons than the background sites (Sites 2 and 4), and correspond with other observed chemistry changes (see 4.3.1, and Akob et al., 2016).

The small ratios (much less than 0.1) of the dissolved hydrocarbons to methane (C_n/C₁), and the lack of an apparent cracking pattern of decreasing ratio with increasing carbon number (Fig. 2c) are not consistent with a thermogenic natural gas (e.g. UOG from the Marcellus Shale) source of these compounds (Taylor et al., 2000). Observed concentrations are likely a combination of air/water equilibration, and chemical and biological activity in the stream and in the groundwater influx that feeds the stream. Akob et al. (2016) show that the microbial community at Sites 7 and 3 contained more anaerobic orders of bacteria (e.g. Desulfuromonadales, Anaerolineales, and Syntrophobacterales) suggesting anaerobic conditions in

the streambed sediments at these sites. In contrast, Site 4 (upstream of the UIC well) was characterized by a more aerobic microbial community (e.g. Rhizobiales, Myxococcales, and Sphingobacteriales). Thus, methanogenic archaea might be producing biological methane at the more anaerobic Sites 7 and 3, compared to the more aerobic upstream Site 4.

4.3 Extractable Hydrocarbons in Water

Extractable hydrocarbons were observed in all water samples at low concentrations; individual compounds most often present at $<1\text{ }\mu\text{g/L}$ (Table 2). Total ion current chromatograms (TICs) of the upstream to downstream sites give an overall view of distributions along the unnamed tributary near the disposal facility and into Wolf Creek, with more peaks (compounds) apparent at Sites 7 and 3 compared to sites upstream and downstream (Fig. 3). Of particular note is the unresolved complex mixture (UCM) in the TIC (20-35 min. retention time in TIC) from Site 7, for the water sample collected in 2013 (Fig. 3b). The UCM is a mixture of hydrocarbons present in such abundance that the GC column was unable to resolve individual compounds, resulting in a “hump” in the chromatogram. The UCM may consist primarily of aliphatic and aromatic hydrocarbons (Isaacman et al., 2012), with aliphatics roughly in the range of C_{15} to C_{27} based on retention time. The UCM at site 7 was not present in sampling in June 2014, possibly due to dilution from heavy rainfall in the week prior to the June sampling (NOAA, 2015). An elevated baseline in the TIC for Site 7 was observed in September 2014 and not observed at the other sites. Fig. S2.3 (Supplemental Material) shows the TIC at Site 7 in September 2014 versus a blank baseline to more clearly illustrate the elevated baseline. This elevated baseline may also represent an unresolved complex mixture of hydrocarbons. It is possible that the closure of the settling pond in February 2014 had resulted in reduced release of extractable hydrocarbons to the creek, causing diminution of the observed UCM. No UCM was observed in TICs of extractable hydrocarbons from water samples at other sites.

Individual organic compounds identified in water samples include some associated with UOG production (Table 2). A sample chromatogram of extractable hydrocarbons from water at Site 3 with identified compounds and internal standards is shown in the Supplemental Material (Fig. S2.1). Compounds identified at Site 3 that are known to be present in UOG wastewater include: tetrahydro-thiophene 1,1-dioxide (present on all three sampling dates), acetophenone, α,α -dimethyl-benzenemethanol, 2-(2-butoxyethoxy)-ethanol, and tris(1-chloro-2-propyl)phosphate (US House of Representatives Committee on Energy and Commerce Minority Staff, 2011). Many of the same, and other compounds that have been identified in UOG wastewater were observed at Site 7, including: tetrahydro-thiophene 1,1-dioxide, acetophenone, α,α -dimethyl-benzenemethanol, tris(1-chloro-2-propyl)phosphate, 4-methylphenol, and 3-ethyl-4-methyl-1H-pyrrole-2,5-dione. Compounds probably not linked to a UOG wastewater source at Sites 3 and 7 include a number of long-chain saturated fatty acids and caffeine. At Site 6A, two compounds (1-methyl-2-pyrrolidinone, and 1-[2-(2-methoxy-1-methylethoxy)-1-methylethoxy]-2-propanol) linked to UOG production were identified. In addition, a compound (1,2,3-triacetoxyp propane) possibly linked to truck activity on the well pad, was identified. The diethyltoluamide (DEET; active ingredient in insect repellent) identified at Site 6 is likely a contaminant from sample collection activity. Water from site 4, located slightly upstream of the UIC well, contained only one identified compound in June 2014 (2,6-dimethoxybenzoquinone). This compound is toxic, but has natural plant and microbial sources and is not known to be linked to UOG wastewater. In September 2014, however, three compounds possibly linked to UOG wastewater were identified: acetophenone, α,α -dimethyl-benzenemethanol, and 2-(2-butoxyethoxy)-ethanol. The presence of these compounds at Site 4 is unexplained, as there are no other indications of UOG wastewater at this site (Akob et al., 2016). Similarly, Site 2 (a tributary of Wolf Creek separated from the UIC well by a mountain ridge) had only one identified compound present in the 2013 sample (octanoic acid, a natural product), but the June 2014 sample had two compounds present at low levels that are linked to UOG wastewater (α,α -dimethyl-benzenemethanol, and 2-(2-butoxyethoxy)-ethanol), as well as 4,4'-(1-methylethylidene)*bis*-phenol (bisphenol A), which is present in some plastics, including drainage pipes (Table 2). All other compounds

at Site 2 in June 2014 were natural products (long-chain saturated fatty acids). The source(s) of the industrial anthropogenic chemicals at Site 2 is/are not certain (field and laboratory blanks did not contain these compounds), however, a private well nearby did contain water with aliphatic and aromatic hydrocarbons suggesting petroleum contamination (Orem, unpublished data). This illustrates the problem of finding unimpacted control sites in populated areas.

Overall, these results indicate that surface water in the creek in the vicinity of the UIC well is contaminated with low levels of organic compounds consistent with a source from UOG wastewater. No UOG-linked contaminants were observed in water further downstream (Wolf Creek) than Site 3. Water chemistry parameters, such as conductivity, and major cation and anion concentrations, have shown increases in elements associated with UOG wastewater downstream of the disposal facility at Sites 7 and 3 (see Akob et al., 2016). Thus, both organic and inorganic geochemistry results indicate the presence of UOG wastewater contaminants immediately downstream of the UIC well.

4.4 Extractable Hydrocarbons in Sediments

The major identified extractable hydrocarbons isolated from sediments and their concentrations at Sites 2, 3, 4, and 7 are shown in Table 3. Site 7 contained the greatest abundance of UOG-linked industrial chemicals, including a number of straight chain, branched, and cyclic alkanes and alkenes found in oil and diesel fuel and many identified in wastewater from UOG production (e.g. octadecane, pentacosane, 1-hexacosene, Z-14-nonacosane, hentriacontane, 2,6,10,14-tetramethylhexadecane, 1,7,11-trimethyl-4-(1-methylethyl)-cyclotetradecane). A number of compounds of natural origin were also identified in the sediments at this site, such as alkanes and aldehydes of plant origin, and derivatives of cholesterol of probable animal origin (Table 3). Perhaps the most unusual feature of the organic compounds in sediments at Site 7 was the presence of numerous halogenated (fluorinated, chlorinated, brominated, and iodinated) hydrocarbons that are not DBPs. Some of these compounds have been observed in wastewater from shale gas wells (e.g. dotriacontyltrifluoroacetate, 1,54-dibromo-tetrapentacontane; US House of

Representatives Committee on Energy and Commerce Minority Staff, 2011), while others are of unknown origin. Fluorinated compounds are often used as flow tracers in drilling in shale plays (Maguire-Boyle and Barron, 2014). However, the source(s) of many of these halogenated compounds is unknown. A significant UCM observed in the TIC of extractable hydrocarbons from sediments at Site 7 was not observed at other sites (Fig. 4).

Sediments at Site 3, which is about 100 m downstream from Site 7, contained a mixture of natural organic substances (e.g. long-chain fatty acids, aldehydes, and phenols) and organic compounds possibly derived from industrial activity and some identified in UOG wastewater, mostly mid-chain alkanes (e.g. tetradecane, octadecane, eicosane). The halogenated hydrocarbons observed in abundance at Site 7 were not observed in sediments from Site 3.

At Site 4, slightly upstream of the UIC well, all of the hydrocarbons identified in the sediments appear to be of natural origin, including long-chain fatty acids likely of microbial origin), plant phenols, and long-chain n-alkanes with an odd/even carbon preference indicative of vascular plant waxes (Eglinton and Hamilton, 1967; Kolattukudy, 1976). One compound, (Z)-9-octadecenamide, does have industrial uses as a corrosion inhibitor, but is also present in natural products. This multiple source issue for many organics makes linking specific organic compounds to an industrial activity (like UOG production) a challenge. Convincing arguments on sources of organic contaminants require demonstrated links to the potential activity producing contaminants (e.g. compound specificity, proximity, hydrologic linkage, downwind of source, etc.), and multiple compounds known to be used in the source activity. Sediments from Site 2 contained a number of alkanes. The higher molecular weight n-alkanes (C_{18} and higher) showed an odd/even carbon preference suggesting a source from vascular plant waxes (Eglinton and Hamilton, 1967; Kolattukudy, 1976). The lower molecular weight n-alkanes (C_8 - C_{16}), however, showed no odd/even carbon predominance and are possibly derived from an oil source. As mentioned earlier, well water from a home approximately 150 m upstream of Site 2 contained a large number of aliphatic and aromatic hydrocarbons consistent with an oil source, but the source of the low molecular weight n-alkanes in

sediments from Site 2 is unknown. Other organic compounds observed in sediments at Site 2 have a likely biological origin from plants (beta-amyrin, stigmast-4-en-3-one). No compounds specifically linked to UOG production were observed in sediments at Site 2.

4.5 Biocides and DBPs

No targeted UOG biocides were detected in any of the water samples, despite the presence of other organic compounds observed in UOG wastewater in water samples from Sites 7 and 3. Many biocides used in UOG production are unstable and biodegradable (Kahrilas et al., 2015), and may not persist in wastewater or natural waters.

DBPs were also not detected in any of the water samples. DBPs are usually produced from precursor organic substances present during chemical treatment of water with chlorine or bromine as a disinfection step. It is possible that none of the produced water being disposed of at this injection well was chemically treated in a way that could produce any DBPs as we could find no records of pretreatment. Furthermore, DBPs are volatile and could be lost from produced water during transport or storage. Halogenated hydrocarbons were detected in sediments (section 4.4), but these are not typically classified as DBPs.

4.6 Toxicology

Water samples were collected for toxicology only in 2013 (August and September). The 24 hour toxicity testing showed no significant acute toxicity at any site (Fig. 5). Water from Site 11 (on a separate tributary of Wolf Creek from the disposal facility) showed excessive growth in the 24 hour toxicity assay (relative to control), and also showed excessive growth in the clonal efficiency assay (SI, Fig. S2.4), which the literature suggests might indicate tumor promoter activity (Eisenbrand et al., 2002). Site 11 is located near a wholesale chemical supplier (water treatment chemicals and dust suppressants), an apartment complex, and a shopping plaza, and may receive runoff from these sources. Contaminants in the water at this site resulting in excessive cell growth are unlikely to be contaminants from the disposal facility. No impact from water at other sites was observed in the clonal efficiency assay (SI, Fig. S2.4). In

the anchorage independent growth assay (SI, Fig. S2.5), no positive response (effect) was observed in cells exposed to water from any sites in this study. Overall, little acute toxicity of water samples to the human cell lines was observed for the assays conducted here. Kassotis et al. (2016) observed endocrine disrupting activities on mammalian cells from exposure to water from Sites 7 and 3 (compared to Site 4), including higher antagonist activity for the estrogen, androgen, progesterone, glucocorticoid, and thyroid hormone receptors. Of note, the samples used in the exposures in the endocrine study (Kassotis et al., 2016) were concentrates of 1 L of stream water eluted from solid phase extraction cartridges, compared to these unconcentrated water samples. In comparing these results with those of Kassotis, et al., it is important to distinguish the different endpoints being collected. The present toxicology assays were designed to detect acute toxicity, of the kind immediately dangerous to life or health (IDLH). EDCs, on the other hand, are chronic toxicants typically detected through receptor binding assays, for instance, the binding or activation of the estrogen/androgen receptor, rather than by cell death. In our experience it is rare to observe IDLH toxicity in environmental samples.

No significant acute toxicity in the 24 hour toxicity assay was observed for any sediment extracts collected in this study (SI, Fig. S2.6). In the anchorage independent growth assay, sediment from Site 3 showed a positive result, while sediment samples from all other sites showed either no change ($=1.0$) or less growth than (significantly <1.0) controls (Fig. 6). Thus, sediment from Site 3, just downstream from the facility (Fig. 1), facilitated the growth of anchorage dependent cells in an abnormal (anchorage independent) manner, and could potentially have tumorigenic or tumor-promoting characteristics (Ke et al., 2004). In the clonal efficiency assay for these sediments (SI, Fig. S2.7), 6 of the samples showed toxicity, while none of the samples showed a clear propensity to act as a cancer promoter, after a theoretical mutational event. However, the clonal efficiency assay of water, vegetable, and soil material from 2013 (August and September) showed a positive effect at Site 11 (located in separate tributary of Wolf Creek from the disposal facility). Therefore, this sample may have tumor promotion, or growth promotion compared to controls.

It should be noted that these assays are focused on acute toxicity only, and the effects of chronic exposure are unknown and not addressed here. All *in vitro* assay results should be confirmed by various methods before they are considered definitive, especially with the use of *in vivo* toxicology testing.

5. Conclusions

An examination of organic substances in water and sediments from a stream near a UIC well for disposal of UOG wastewater and nearby drainages was conducted to examine potential contamination. Toxicology using several different assays was also conducted on water and sediment from the same sites as a preliminary evaluation of toxicity and excessive cell growth. Various light and heavier hydrocarbons, consistent with organic chemicals present in UOG wastewater, had elevated concentrations in stream water near the UIC well, including: acetophenone, α , α -dimethyl-benzenemethanol, 2-(2-butoxyethoxy)-ethanol, tris(1-chloro-2-propyl)phosphate, tetrahydro-thiophene, 1,1-dioxide and others (US House of Representatives Committee on Energy and Commerce Minority Staff, 2011; Maguire-Boyle and Barron, 2014). Stream water at Sites 7 and 3 (just downstream of the well) were the most impacted. A UCM was observed in the GC/MS total ion current chromatogram of extractable hydrocarbons from Site 7 (Fig. 3), indicating the presence of a complex mixture of a large number of hydrocarbons that are unidentified.

Akob et al. (2016) also observed changes in the inorganic chemistry of the water at Sites 3 and 7 consistent with impacts from the UIC well. Conductivity was ten times higher at Sites 3 and 7 compared to other sites more representative of background chemistry in this creek. Akob et al. (2016) also showed that Sites 3 and 7 had elevated concentrations of Cl^- , Ca^{2+} , Na^+ , Sr^{2+} , and Ba^{2+} , which are dissolved ions that have been demonstrated to be associated with UOG wastewater (Rowan, 2011; Haluszczak et al., 2013; Brantley et al., 2014; Harkness et al., 2015; Akob et al., 2015; Shih et al., 2015). Both organic and inorganic chemical data from the surface water point to an impact of UOG wastewater on the creek immediately downstream of the UIC well. The impact on surface water, however, appears to be small as concentrations of organic compounds consistent with a UOG wastewater source are low (Table 2), and

the amount of wastewater brine needed to produce the observed Cl^- and Br^- concentrations is one part wastewater in one thousand parts surface water (Akob et al., 2016). Furthermore, no indication of organic or inorganic substances consistent with a UOG wastewater source were identified further downstream in Wolf Creek (below Site 3), or in other tributaries (though organic contaminants not linked to UOG wastewater were observed).

Extractable hydrocarbons from streambed sediments at Sites 3 and 7 further reinforce the observation of UOG wastewater impact just downstream of the UIC well. The total ion current chromatogram of extractable hydrocarbons from Site 7 had a significant UCM, indicating the presence of a complex mix of hydrocarbons. In addition, a number of organic compounds known to be present in UOG wastewater were observed in the sediments at Site 7, and to a lesser extent at Site 3 (Table 3). Numerous halogenated hydrocarbons (fluorinated, chlorinated, brominated, and iodinated) were also present in sediments from Site 7. While some of the fluorinated and brominated hydrocarbons have been observed in UOG wastewater, the origin of others is unknown, but unlikely to be from natural local sources. Sediments from Site 4, upstream of the UIC well, contained hydrocarbons consistent with natural sources only, while sediments from Site 2 (background stream) contained alkanes with a possible diesel fuel source and compounds of biological origin.

There was no significant acute toxicity observed in any of the samples from raw water samples tested in this study. Sporadic growth promotion was observed from samples at Sites 3 (sediment) and 11 (water), but there was no gradient of toxicologic effect seen with distance from the UIC site, nor along the Wolf Creek waterway in these assays. Whereas *in vitro* assays are of limited utility in predicting human health effects, they can be useful screening tools to determine whether precautions or site mitigation should be undertaken. The present limited results do not support a finding of an acute health hazard, but increased growth promotion (e.g. water from Site 11 and sediment from Site 3) may indicate the potential for long-term effects.

Akob et al. (2016) have shown that impacted Sites 7 and 3 had a less diverse and more anaerobic microbial community compared to other sites (more aerobic) along the stream. Higher light hydrocarbon concentrations with a biological signature (e.g. low C_n/C_1 ratio) at Sites 7 and 3 may reflect microbial gas produced as a consequence of the more anaerobic community structure at these sites supporting methanogenesis.

Overall, results indicate that the upper portions of the creek just downstream of the UIC well (Sites 7 and 3) are contaminated with organic substances, many of which are used in UOG production. Contamination appears to be largely confined, at this time, to the upper reaches of the unnamed tributary to Wolf Creek near the UIC facility. While the composition of the organic chemicals present is consistent with compounds present in UOG wastewater, some of these compounds may also be present in other types of wastewater (e.g. coalbed methane produced water). The proximity of contamination to the UIC well is compelling, and suggests that sources such as wastewater spills on the pad, leakage or overflow from a settling pond (previously on this site and now filled in), and other activities related to injection of the wastewater (e.g. leakage of fluids from trucks) may contribute to the observed contamination. A specific point source or pathway of the contaminants to the surface water from the UIC well pad, however, was not demonstrated as part of this study. The toxicity of the wastewater from UOG production requires that operators involved in disposal and long-term storage of these wastes take extra precautions to avoid even small scale contamination, as observed here.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Table 1. Sampling site locations, descriptions and general geochemistry.

Site	Description	NPDOC (mg/L)			TC %	TN %	TS %
		Sep-13	Jun-14	Sep-14	dry wt.	dry wt.	dry wt.
2	control or background creek separated by ridge from UIC well	2.7	1.4	NC ¹	1.57	0.14	0.031
3	about 200 m downstream of UIC well at junction of two unnamed drainages	3.9	2.5	3.3	1.28	0.18	0.033
4	unnamed drainage upstream of UIC well	NC	1.1	1.6	2.20	0.22	0.041
5	drainage near UIC well; on same level as well pad	NC	3.3	NC	NC	NC	NC
6	adjacent to the injection well shed	NC	2.2	NC	5.43	0.26	<1
6A	drainage flowing under filled-in settling pond at UIC well	NC	NC	NC	3.13	0.22	0.074
7	drainage downstream of UIC well and directly across from settling pond	7.4	2.0	3.0	1.28	0.16	0.032
8	Dempsey Branch/Laurel Creek off Dempsey Road Fayetteville, WV	2.1	NC	NC	2.24	0.12	0.049
9	Wolf Creek at Town Center Park, Fayetteville, WV	4.1	NC	NC	2.04	0.15	0.032
10	Wolf Creek at Broadway Avenue Oak Hill, WV	4.1	NC	NC	0.34	0.12	0.023
11	unnamed drainage in Oak Hill, WV not hydrologically connected to UIC well	3.6	NC	NC	NC	NC	NC
12	House Branch tributary of Wolf Creek Maple Ave. Fayetteville, WV	2.9	NC	NC	NC	NC	NC

¹NC = not collected

Table 2. Organic compounds identified in water samples from sites 3, 4, 6A, and 7 in September 2013, June 2014, and September 2014.

Site	Date	Compounds	Concentration ¹	Sources and Industrial Uses
			(µg/L)	
2	9/14/2013	octanoic acid, methyl ester	0.11	C ₈ fatty acid methylester, biological sources
	6/17/2014	α,α-dimethyl-benzenemethanol*	0.26	Component of VE-B hardener used to stabilize concrete
		2-(2-butoxyethoxy)-ethanol*	0.28	Solvent: paint, hydraulic fracturing, drill stabilizers, oil dispersants
		dodecanoic acid	0.84	C ₁₂ fatty acid
		tetradecanoic acid	0.58	C ₁₄ fatty acid
		hexadecanoic acid	0.84	C ₁₆ fatty acid
		octadecanoic acid	0.41	C ₁₈ fatty acid
		4,4'-(1-methylethylidene)bis-phenol	2.6	Bisphenol-A; plastics, electronics, investment casting
3	9/14/2013	tetrahydrothiophene, 1,1-dioxide (sulfolane)*	4.0	solvent used to purify natural gas
		caffeine	0.28	foodstuffs, coffee, plant sources, may be present in urine
	6/17/2014	acetophenone	0.09	resin feedstock; found in UOG wastewater; natural sources as well
		α,α-dimethyl-benzenemethanol*	0.34	Component of VE-B hardener used to stabilize concrete.
		2-(2-butoxyethoxy)-ethanol*	0.54	Solvent: paint, hydraulic fracturing, drill stabilizers, oil dispersants
		tetrahydrothiophene, 1,1-dioxide (sulfolane)*	0.48	solvent used to purify natural gas
		tris(1-chloro-2-propyl)phosphate*	0.27	Flame retardant used in paint and polyurethane foam.
	9/16/2014	acetophenone*	0.23	resin feedstock; found in UOG wastewater; natural sources as well
		α,α-dimethyl-benzenemethanol*	0.33	Component of VE-B hardener used to stabilize concrete.
		tetrahydro-thiophene, 1,1-dioxide (sulfolane)*	1.0	solvent used to purify natural gas
		dodecanoic acid	0.36	C ₁₂ fatty acid
		hexadecanoic acid	0.43	C ₁₆ fatty acid
4	6/17/2014	2,6-dimethoxybenzoquinone	0.04	natural sources from plants; anti microbial activity
	9/16/2014	acetophenone*	1.3	resin feedstock; found in UOG wastewater; natural sources as well
		α,α-dimethyl-benzenemethanol*	1.5	Component of VE-B hardener used to stabilize concrete.
		2-(2-butoxyethoxy)-ethanol*	0.1	Solvent: paint, hydraulic fracturing, drill stabilizers, oil dispersants
		benzothiazole	0.1	N and S heterocyclic; natural and industrial sources
		decanoic acid	0.16	C ₁₀ fatty acid
		dodecanoic acid	0.11	C ₁₂ fatty acid
		tetradecanoic acid	0.19	C ₁₄ fatty acid

6A	6/17/2014	1-methyl-2-pyrrolidinone*	0.02	Solvent, strip H ₂ S, pharmaceuticals, lithium batteries.
		1-[2-(2-methoxy-1-methylethoxy)-1-methylethoxy]-2-propanol	0.01	Diesel engine antifreeze.
		1,2,3-triacetoxyp propane (triacetin)	0.02	Flavoring solvent, fuel additive (antiknock), plasticizer
		diethyltoluamide	0.02	DEET, active ingredient in insect repellent.
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7	9/14/2014	octanoic acid	1.5	C ₈ fatty acid
		tetrahydrothiophene, 1,1-dioxide (sulfolane)*	1.5	solvent used to purify natural gas
		3-ethyl-4-methyl-1H-pyrrole-2,5-dione*	0.16	present in wastewater from UOG production
6/17/2014		tris(1-chloro-2-propyl)phosphate*	0.3	Flame retardant used in paint and polyurethane foam.
		cis-9-hexadecenoic acid	0.04	Omega-7 monounsaturated fatty acid - biological sources.
		hexadecanoic acid	0.03	Common fatty acid found in plants/animals/microbes.
9/16/2014		acetophenone*	0.73	resin feedstock; found in UOG wastewater; natural sources as well
		4-methylphenol (<i>p</i> -cresol)	0.25	present in concrete
		α,α -dimethyl-benzenemethanol*	1.1	Component of VE-B hardener used to stabilize concrete.
		tetrahydrothiophene, 1,1-dioxide (sulfolane)*	2	solvent used to purify natural gas
		decanoic acid	0.2	C ₁₀ fatty acid
		tetradecanoic acid	0.19	C ₁₄ fatty acid
		hexadecanoic acid	0.13	C ₁₆ fatty acid

¹Estimated concentrations; see text for details

*Organic compounds linked to UOG production and/or present in UOG wastewater

Table 3. Compounds identified in streambed sediments from USGS sites along Wolf Creek, WV (June 2014).

Site	Compounds	Concentration $\mu\text{g/g}^1$	Notes
3	tridecanoic acid	27	common fatty acid from microbial sources
	tetradecane*	2.0	C ₁₄ alkane present in many solvents; in produced water from UOG
	tetradecanoic acid	13	common fatty acid from microbial sources
	pentadecanoic acid	2.1	common fatty acid from microbial sources
	octadecane*	15	C ₁₈ alkane; present in wastewater from UOG production
	octadecanal, two isomers	33	C ₁₈ aldehyde; insect pheromone
	eicosane, two isomers*	10.3	C ₂₀ hydrocarbon in oil; in produced water from shale gas wells
	(E)-3-eicosene*	7.5	alkene in some plants; identified in produced water from UOG
	benzocycloheptatriene	3.0	C ₁₁ hydrocarbon; industrial use as organometallic
	benzeneacetic acid	2.7	natural product in plants and animals
	4-methyl-phenol (p-cresol)	9.7	present in coal tar, intermediate in antioxidant manufacture
	hexadecamethyl-octasiloxane	13	Contaminant from analytical column used in GC/MS
	elemental sulfur	18	reduced sulfur species - microbial indicator of anaerobic conditions
	cyclic octaatomic sulfur	56	reduced sulfur species - microbial indicator of anaerobic conditions
4	tetradecanoic acid	2.7	long-chain fatty acid - likely microbial source
	pentadecanoic acid	2.4	long-chain fatty acid - likely microbial source
	n-hexadecanoic acid	16	long-chain fatty acid - likely microbial source
	cis-9-hexadecenoic acid	3.3	long-chain fatty acid - likely microbial source
	octadecanal	6.5	aldehyde of octadecanoic acid - microbial byproduct/insect pheromone
	eicosane	12	C ₂₀ n-alkane; present in seeds and other plant components
	4-methyl-phenol (p-cresol)	5.0	present in coal tar, intermediate in antioxidant manufacture
	α -tocopherol (vitamin E)	4.9	methylated phenol of plant or animal origin
	(Z)-9-octadecenamide	11	industrial uses: slip agent, lubricant, corrosion inhibitor
	elemental sulfur	10	reduced sulfur species - microbial origin - indicator of anaerobic conditions
7	cyclic octaatomic sulfur	39	reduced sulfur species - microbial origin - indicator of anaerobic conditions
	1-bromo-11-iodoundecane	1.9	C ₁₁ n-alkane with bromine and iodine; unknown source
	octadecane*	10	C ₁₈ n-alkane; natural/industrial sources; in hydraulic fracturing fluid
	1-chloro-octadecane	2.0	halogenated hydrocarbon; unknown sources
	1-iodo-octadecane*	7.1	unknown; possible marine origin from shale
	eicosane*	7.1	C ₂₀ n-alkane; present in seeds and other plant components

1-chloroeicosane	4.3	halogenated hydrocarbon; unknown sources
pentacosane*	38	C ₂₅ hydrocarbon found in diesel fuel
1-hexacosene, two isomers*	4.8	C ₂₆ unsaturated hydrocarbon, common component of oil
Z-14-nonacosane, ten isomers*	42	C ₂₉ hydrocarbon found in diesel fuel and in some plant oils
hentriacontane, two isomers*	53	C ₃₁ n-alkane; natural sources; in hydraulic fracturing fluids
2,6,10,14-tetramethyl-hexadecane (phytane)	21	common petroleum biomarker
1,7,11-trimethyl-4-(1-methylethyl)-cyclotetradecane, nine isomers*	51	cyclic hydrocarbon in oil; in produced water from UOG
11,13-dimethyl-12-tetradecen-1-ol acetate	2.7	found in leaf extract and insects
benzeneacetic acid	3.1	plant hormone; insect antimicrobial agent
4-hydroxy-benzaldehyde	5.2	found in plants; aromatic substance
3-hydroxy-4-methoxy-benzaldehyde	4.7	isomer of vanillin from vascular plants
(all-E)-oxirane compound ²	4.4	present in herbaceous plants and flowers; found in cigarette smoke
2-methylene-, (3.beta.,5.alpha.)-cholestan-3-ol	17	cholesterol derivative; probable animal source
2-methylene-, (3.beta.,5.alpha.)-cholestan-3-ol	22	cholesterol derivative; probable animal source
friedelan-3-one	37	triterpene found in vascular plants
1-heneicosyl formate	15	ester of unknown origin; has been found in human bodyburden
heptacosyl acetate*	44	C ₂₇ alkane bonded to acetate; use as viscosity modifier in UOG
triacontyltrifluoroacetate	2.1	C ₃₀ alkane bonded to an acetate with 3 fluorines; unknown sources
triacontylheptafluorobutyrate, three isomers	8.4	C ₃₀ alkane bonded to a butyrate with 7 fluorines; unknown sources
dotriacontylheptafluorobutyrate	3.0	C ₃₂ alkane bonded to a butyrate with 7 fluorines; unknown sources
dotriacontyltrifluoroacetate	1.7	C ₃₂ alkane bonded to an acetate with 3 fluorines; unknown sources
dotriacontylpentafluoropropionate, five isomers	11.2	C ₃₂ alkane bonded to a propionate with 5 fluorines; unknown sources C ₃₂ alkane/trifluoroacetate (TFA); TFA used as breaker in hydraulic fracturing
dotriacontyltrifluoroacetate, two isomers*	8.6	
tetratriacontylpentafluoropropionate	2.6	C ₃₄ alkane bonded to a propionate with 5 fluorines; unknown sources
tetratriacontyltrifluoroacetate, three isomers	22	C ₃₄ alkane bonded to an acetate with 3 fluorines; unknown sources
octatriacontyltrifluoroacetate*	20	C ₃₈ alkane with 3 fluorines; present in some plants; used as corrosion inhibitor
octatriacontylpentafluoropropionate*	18	C ₃₈ alkane with 5 fluorines; present plants; corrosion inhibitor for steel
1,54-dibromo-tetrapentacontane, two isomers*	63	C ₅₄ hydrocarbon with bromines at ends; in UOG wastewater
11,12-dibromo-tetradecan-1-ol acetate	24	C ₁₄ alkane with bromines at 11 and 12 positions; unknown sources

¹concentration as µg compound per g of dry sediment

*Organic compounds used in UOG production and/or present in UOG wastewater

²2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-oxirane