

REFERENCE LIST

EXHIBIT C

(Akob 2016) Akob DM, Mumford AC, Orem WH, Engle MA, Klinges JG, Kent DB, et al. Wastewater disposal from unconventional oil and gas development degrades stream quality at a West Virginia injection facility. Environ Sci Technol 2016;50:5517–25

The development of unconventional oil and gas (UOG) resources has rapidly increased in recent years; however, the environmental impacts and risks are poorly understood. A single well can generate millions of liters of wastewater, representing a mixture of formation brine and injected hydraulic fracturing fluids. One of the most common methods for wastewater disposal is underground injection; we are assessing potential risks of this method through an intensive, interdisciplinary study at an injection disposal facility in West Virginia. In June 2014, waters collected downstream from the site had elevated specific conductance (416 $\mu\text{S}/\text{cm}$) and Na, Cl, Ba, Br, Sr, and Li concentrations, compared to upstream, background waters (conductivity, 74 $\mu\text{S}/\text{cm}$). Elevated TDS, a marker of UOG wastewater, provided an early indication of impacts in the stream. Wastewater inputs are also evident by changes in $^{87}\text{Sr}/^{86}\text{Sr}$ in streamwater adjacent to the disposal facility. Sediments downstream from the facility were enriched in Ra and had high bioavailable Fe(III) concentrations relative to upstream sediments. Microbial communities in downstream sediments had lower diversity and shifts in composition. Although the hydrologic pathways were not able to be assessed, these data provide evidence demonstrating that activities at the disposal facility are impacting a nearby stream and altering the biogeochemistry of nearby ecosystems.

(Cozzarelli 2016) Cozzarelli IM, Skalak KJ, Kent DB, Engle MA, Benthem A, Mumford AC, Haase K, Farag A, Harper D, Nagel SC, et al. Environmental signatures and effects of an oil and gas wastewater spill in the Williston Basin, North Dakota. Sci Total Environ doi: 10.1016/j.scitotenv.2016.11.157.

Wastewaters from oil and gas development pose largely unknown risks to environmental resources. In January 2015, 11.4 M L (million liters) of wastewater (300 g/L TDS) from oil production in the Williston Basin was reported to have leaked from a pipeline, spilling into Blacktail Creek, North Dakota. Geochemical and biological samples were collected in February and June 2015 to identify geochemical signatures of spilled wastewaters as well as biological responses along a 44-km river reach. February water samples had elevated chloride (1030 mg/L) and bromide (7.8 mg/L) downstream from the spill, compared to upstream levels (11 mg/L and < 0.4 mg/L, respectively). Lithium (0.25 mg/L), boron (1.75 mg/L) and strontium (7.1 mg/L) were present downstream at 5–10 times upstream concentrations. Light hydrocarbon measurements indicated a persistent thermogenic source of methane in the stream. Semi-volatile hydrocarbons indicative of oil were not detected in filtered samples but low levels, including tetramethylbenzenes and di-methylnaphthalenes, were detected in unfiltered water samples downstream from the spill. Labile sediment-bound barium and strontium concentrations (June 2015) were higher downstream from the Spill Site. Radium activities in sediment downstream from the Spill Site were up to 15 times the upstream activities and, combined with Sr isotope ratios, suggest contributions from the pipeline fluid and support the conclusion that elevated concentrations in Blacktail Creek water are from the leaking pipeline. Results from June 2015 demonstrate the persistence of wastewater effects in Blacktail Creek several months after remediation efforts started. Aquatic health effects were observed in June 2015; fish bioassays

showed only 2.5% survival at 7.1 km downstream from the spill compared to 89% at the upstream reference site. Additional potential biological impacts were indicated by estrogenic inhibition in downstream waters. Our findings demonstrate that environmental signatures from wastewater spills are persistent and create the potential for long-term environmental health effects.

(Gross 2012) Gross SA, Avens HJ, Banducci AM, Sahmel J, Panko JM, Tvermoes BE. 2013. Analysis of BTEX groundwater concentrations from surface spills associated with hydraulic fracturing operations. Air Waste Manage Assoc 63(4):424-432, doi: 10.1080/10962247.2012.759166.

Concerns have arisen among the public regarding the potential for drinking-water contamination from the migration of methane gas and hazardous chemicals associated with hydraulic fracturing and horizontal drilling. However, little attention has been paid to the potential for groundwater contamination resulting from surface spills from storage and production facilities at active well sites. We performed a search for publically available data regarding groundwater contamination from spills at ULS. drilling sites. The Colorado Oil and Gas Conservation Commission (COGCC) database was selected for further analysis because it was the most detailed. The majority of spills were in Weld County, Colorado, which has the highest density of wells that used hydraulic fracturing for completion, many producing both methane gas and crude oil. We analyzed publically available data reported by operators to the COGCC regarding surface spills that impacted groundwater. From July 2010 to July 2011, we noted 77 reported surface spills impacting the groundwater in Weld County, which resulted in surface spills associated with less than 0.5% of the active wells. The reported data included groundwater samples that were analyzed for benzene, toluene, ethylbenzene, and xylene (BTEX) components of crude oil. For groundwater samples taken both within the spill excavation area and on the first reported date of sampling, the BTEX measurements exceeded National Drinking Water maximum contaminant levels (MCLs) in 90, 30, 12, and 8% of the samples, respectively. However, actions taken to remediate the spills were effective at reducing BTEX levels, with at least 84% of the spills reportedly achieving remediation as of May 2012. Our analysis demonstrates that surface spills are an important route of potential groundwater contamination from hydraulic fracturing activities and should be a focus of programs to protect groundwater.

(Hoelzer 2016) Hoelzer K, Sumner AJ, Karatum O, Nelson RK, Drollette BD, O'Connor MP, D'Ambro EL, Getzinger GJ, Ferguson PL, Reddy CM, et al. 2016. Indications of transformation products from hydraulic fracturing additives in shale-gas wastewater. Environ Sci Technol doi: 10.1021/acs.est.6b00430.

Unconventional natural gas development (UNGD) generates large volumes of wastewater, the detailed composition of which must be known for adequate risk assessment and treatment. In particular, transformation products of geogenic compounds and disclosed additives have not been described. This study investigated six Fayetteville Shale wastewater samples for organic composition using a suite of one- and two-dimensional gas chromatographic techniques to capture a broad distribution of chemical structures. Following the application of strict compound-identification-confidence criteria, we classified compounds according to their putative origin. Samples displayed distinct chemical distributions composed of typical geogenic substances (hydrocarbons and hopane biomarkers), disclosed UNGD additives (e.g., hydrocarbons, phthalates such as diisobutyl phthalate, and radical initiators such as

azobis(isobutyronitrile)), and undisclosed compounds (e.g., halogenated hydrocarbons, such as 2-bromohexane or 4-bromoheptane). Undisclosed chloromethyl alkanoates (chloromethyl propanoate, pentanoate, and octanoate) were identified as potential delayed acids (i.e., those that release acidic moieties only after hydrolytic cleavage, the rate of which could be potentially controlled), suggesting they were deliberately introduced to react in the subsurface. In contrast, the identification of halogenated methanes and acetones suggested that those compounds were formed as unintended byproducts. Our study highlights the possibility that UNGD operations generate transformation products and underscores the value of disclosing additives injected into the subsurface.

(Kassotis 2013) Kassotis CD, Tillitt DE, Davis JW, Hormann AM, Nagel SC. 2014. Estrogen and androgen receptor activities of hydraulic fracturing chemicals and surface and ground water in a drilling-dense region. *Endocrinology* 155 (3): 897-907, doi:10.1210/en.2013-1697.

The rapid rise in natural gas extraction using hydraulic fracturing increases the potential for contamination of surface and ground water from chemicals used throughout the process. Hundreds of products containing more than 750 chemicals and components are potentially used throughout the extraction process, including more than 100 known or suspected endocrine-disrupting chemicals. We hypothesized that a selected subset of chemicals used in natural gas drilling operations and also surface and ground water samples collected in a drilling-dense region of Garfield County, Colorado, would exhibit estrogen and androgen receptor activities. Water samples were collected, solid-phase extracted, and measured for estrogen and androgen receptor activities using reporter gene assays in human cell lines. Of the 39 unique water samples, 89%, 41%, 12%, and 46% exhibited estrogenic, antiestrogenic, androgenic, and antiandrogenic activities, respectively. Testing of a subset of natural gas drilling chemicals revealed novel antiestrogenic, novel antiandrogenic, and limited estrogenic activities. The Colorado River, the drainage basin for this region, exhibited moderate levels of estrogenic, antiestrogenic, and antiandrogenic activities, suggesting that higher localized activity at sites with known natural gas-related spills surrounding the river might be contributing to the multiple receptor activities observed in this water source. The majority of water samples collected from sites in a drilling-dense region of Colorado exhibited more estrogenic, antiestrogenic, or antiandrogenic activities than reference sites with limited nearby drilling operations. Our data suggest that natural gas drilling operations may result in elevated endocrine-disrupting chemical activity in surface and ground water.

(Mrdjen 2016) Mrdjen, Igor & Lee, Jiyoung (2016) High volume hydraulic fracturing operations: potential impacts on surface water and human health, *International Journal of Environmental Health Research*, 26:4, 361-380, DOI: 10.1080/09603123.2015.1111314

High volume, hydraulic fracturing (HVHF) processes, used to extract natural gas and oil from underground shale deposits, pose many potential hazards to the environment and human health. HVHF can negatively affect the environment by contaminating soil, water, and air matrices with potential pollutants. Due to the relatively novel nature of the process, hazards to surface waters and human health are not well known. The purpose of this article is to link the impacts of HVHF operations on surface water integrity, with human health consequences. Surface water contamination risks include: increased structural failure rates of unconventional wells, issues

with wastewater treatment, and accidental discharge of contaminated fluids. Human health risks associated with exposure to surface water contaminated with HVHF chemicals include increased cancer risk and turbidity of water, leading to increased pathogen survival time. Future research should focus on modeling contamination spread throughout the environment, and minimizing occupational exposure to harmful chemicals.

(Rodgers 2015) Rogers J.D., Burke T.L., Osborn S.G., Ryan J.N. A framework for identifying organic compounds of concern in hydraulic fracturing fluids based on their mobility and persistence in groundwater. Environ. Sci. Technol. Lett., 2 (2015), pp. 158–164

We developed a screening framework for identifying organic components of hydraulic fracturing fluid with increased probability of exposure via groundwater based on mobility, persistence, toxicity, and frequency of use. Of 996 organic fracturing fluid compounds identified by the U.S. Environmental Protection Agency and FracFocus for four states, data were available to perform an initial screening of 659 compounds for sufficient mobility and persistence to reach a water well under fast and slow groundwater transport scenarios. For the fast transport scenario, 15 compounds identified on at least 50 FracFocus reports were predicted to have an elevated exposure potential, which was defined as $\geq 10\%$ of the initial concentration remaining at a transport distance of 94 m, the average setback distance in the United States. Of these 15 compounds, two were identified on $>20\%$ of FracFocus reports (naphthalene and 2-butoxyethanol), four were compounds identified on $>5\%$ of reports, and three had health-based standards.

(Yao 2015) Yao Y, Chen T, Shen SS, Niu Y, DesMarais TL, Linn R, Saunders E, Fan Z, Lioy P, Kluz T, et al. 2015. Malignant human cell transformation of Marcellus Shale gas drilling flow back water. Toxicol Appl Pharmacol 288(1):121-130, doi: 10.1016/j.taap.2015.07.011.

The rapid development of high-volume horizontal hydraulic fracturing for mining natural gas from shale has posed potential impacts on human health and biodiversity. The produced flow back waters after hydraulic stimulation are known to carry high levels of saline and total dissolved solids. To understand the toxicity and potential carcinogenic effects of these wastewaters, flow back waters from five Marcellus hydraulic fracturing oil and gas wells were analyzed. The physicochemical nature of these samples was analyzed by inductively coupled plasma mass spectrometry and scanning electron microscopy/energy dispersive X-ray spectroscopy. A cytotoxicity study using colony formation as the endpoint was carried out to define the LC_{50} values of test samples using human bronchial epithelial cells (BEAS-2B). The BEAS-2B cell transformation assay was employed to assess the carcinogenic potential of the samples. Barium and strontium were among the most abundant metals in these samples and the same metals were found to be elevated in BEAS-2B cells after long-term treatment. BEAS-2B cells treated for 6 weeks with flow back waters produced colony formation in soft agar that was concentration dependent. In addition, flow back water-transformed BEAS-2B cells show better migration capability when compared to control cells. This study provides information needed to assess the potential health impact of post-hydraulic fracturing flow back waters from Marcellus Shale natural gas mining.

(Yost 2016) Yost, Erin E., et al. "Estimating the Potential Toxicity of Chemicals Associated with Hydraulic Fracturing Operations Using Quantitative Structure–Activity Relationship Modeling." *Environmental science & technology* 50.14 (2016): 7732-7742.

The United States Environmental Protection Agency (EPA) identified 1173 chemicals associated with hydraulic fracturing fluids, flowback, or produced water, of which 1026 (87%) lack chronic oral toxicity values for human health assessments. To facilitate the ranking and prioritization of chemicals that lack toxicity values, it may be useful to employ toxicity estimates from quantitative structure–activity relationship (QSAR) models. Here we describe an approach for applying the results of a QSAR model from the TOPKAT program suite, which provides estimates of the rat chronic oral lowest-observed-adverse-effect level (LOAEL). Of the 1173 chemicals, TOPKAT was able to generate LOAEL estimates for 515 (44%). To address the uncertainty associated with these estimates, we assigned qualitative confidence scores (high, medium, or low) to each TOPKAT LOAEL estimate, and found 481 to be high-confidence. For 48 chemicals that had both a high-confidence TOPKAT LOAEL estimate and a chronic oral reference dose from EPA's Integrated Risk Information System (IRIS) database, Spearman rank correlation identified 68% agreement between the two values (permutation p-value = 1×10^{-11}). These results provide support for the use of TOPKAT LOAEL estimates in identifying and prioritizing potentially hazardous chemicals. High-confidence TOPKAT LOAEL estimates were available for 389 of 1026 hydraulic fracturing-related chemicals that lack chronic oral RfVs and OSFs from EPA-identified sources, including a subset of chemicals that are frequently used in hydraulic fracturing fluids.

Wastewater Disposal from Unconventional Oil and Gas Development Degrades Stream Quality at a West Virginia Injection Facility

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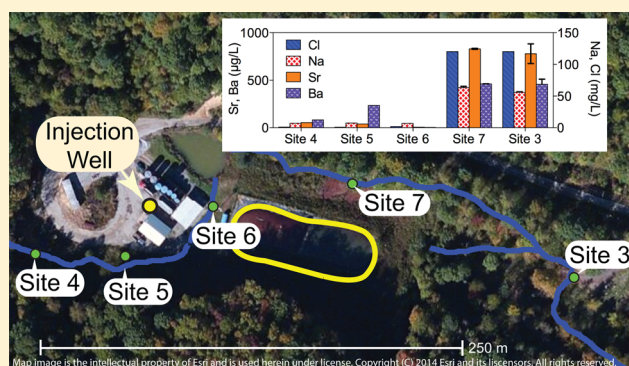
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S Supporting Information

ABSTRACT: The development of unconventional oil and gas (UOG) resources has rapidly increased in recent years; however, the environmental impacts and risks are poorly understood. A single well can generate millions of liters of wastewater, representing a mixture of formation brine and injected hydraulic fracturing fluids. One of the most common methods for wastewater disposal is underground injection; we are assessing potential risks of this method through an intensive, interdisciplinary study at an injection disposal facility in West Virginia. In June 2014, waters collected downstream from the site had elevated specific conductance (416 $\mu\text{S}/\text{cm}$) and Na, Cl, Ba, Br, Sr, and Li concentrations, compared to upstream, background waters (conductivity, 74 $\mu\text{S}/\text{cm}$). Elevated TDS, a marker of UOG wastewater, provided an early indication of impacts in the stream. Wastewater inputs are also evident by changes in $^{87}\text{Sr}/^{86}\text{Sr}$ in streamwater adjacent to the disposal facility. Sediments downstream from the facility were enriched in Ra and had high bioavailable Fe(III) concentrations relative to upstream sediments. Microbial communities in downstream sediments had lower diversity and shifts in composition. Although the hydrologic pathways were not able to be assessed, these data provide evidence demonstrating that activities at the disposal facility are impacting a nearby stream and altering the biogeochemistry of nearby ecosystems.



INTRODUCTION

Increasing demand for fossil fuels and depletion of traditional oil and gas reservoirs has driven the development of shale gas, coal-bed methane (CBM), and other unconventional oil and gas (UOG) resources using technologies such as directional drilling and hydraulic fracturing e.g., ref 1. These resources are an important part of the United States' energy resource portfolio. While CBM production is estimated to remain at current levels of national gas production (8%), development of shale gas is estimated to account for at least 50% of all natural gas produced in the U.S. by 2040.² Production of UOG resources results in liquid (e.g., produced waters or wastewaters), solid (e.g., drill cuttings), and gaseous wastes, which pose unknown risks and potential consequences to the quality of atmospheric, groundwater, surface water, soil, and environmental resources.

Wastewaters from UOG production are mixtures of residual fluids used to complete the well and formation brine.^{1,3–7} Initially, these wastewaters reflect the composition of the injected fluid, then over time, the chemistry shifts to reflect the chemical composition of the formation, e.g.,⁵ Wastewaters from the Marcellus Shale typically have elevated total dissolved

solids (TDS, up to 300 000 mg/L),^{5,8–10} variable concentrations of organic compounds,^{6,11} and naturally occurring radioactive material (NORM¹²). An average well producing from the Marcellus Shale yields approximately 5 million liters of wastewater over its lifetime; these large volumes of returned fluids, with complex chemistries, present water management challenges. The chemistry can affect the suitability of wastewaters for reuse or the strategy for disposal.^{3,4,13,14}

UOG wastewaters are managed in a variety of ways including treatment and reuse for new well completions,¹⁵ disposal through publicly owned or commercial wastewater treatment plants,^{14,15} or disposal in Class II underground injection control (UIC) wells.^{4,14,15} Class II disposal wells are estimated to be disposing of over 2 billion gallons of wastewater from oil and gas operations every day.¹⁶ They are located across the United States and there are approximately 36 000 Class II UIC wells handling wastewaters from UOG operations.¹⁶ Impacts on

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environmental health from accidental or intentional releases during handling, disposal, treatment, or reuse are poorly documented, with few reports in the literature.¹⁷ Potential pathways for wastewater to enter surface water or groundwater include: (1) releases from pipelines or tanker trucks transporting fluids, (2) leakage from wastewater storage ponds through compromised liners and overflows from the ponds, or (3) migration of the fluids through the subsurface at the injection depth or through failed injection well casings. Research is needed to examine the potential impacts of wastewater releases on environmental health, which are likely to accelerate with the growing pace of UOG development.

Near Lochgelly, West Virginia, wastewaters from UOG development are disposed of in a Class II UIC well. A small stream runs through the facility and provides an opportunity to sample surface water and sediments near a wastewater disposal operation (Figure 1). This study is a part of a larger effort by the US Geological Survey (USGS) and university researchers to quantify biogeochemical and human health changes resulting from UOG wastewater disposal.¹⁸ Here, we used key field and inorganic chemical signatures, as well as microbial community compositions, to identify UOG wastewater impacts to stream

biogeochemistry by characterizing differences between sites within the disposal facility and background sites located upstream or in a separate drainage with no known inputs from oil and gas development. Although the pathway of contaminants from the disposal facility to the stream could not be assessed, these results clearly demonstrate that wastewater handling practices at the site affect stream and sediment quality.

MATERIALS AND METHODS

Site Description. The study area is located near a UOG wastewater UIC facility (disposal facility) in central West Virginia (Figures 1 and S10 of the Supporting Information). The disposal well was originally drilled in 1981 as a natural gas production well to a depth of 990.6 m. The well was reworked as a Class II injection well in 2002, with fluids injected into a sandstone reservoir at a depth of 792.5 m. In addition to the disposal well, the facility has brine storage tanks, an access road, and two small, lined impoundment ponds. Until the spring of 2014, a portion of the wastewaters were temporarily stored in the holding ponds prior to injection to allow for settling of particulates that could potentially clog the pore space in the receiving reservoir. The ponds operated from 2002 to spring 2014 when they were removed and the area recontoured and planted with grass.

A certified record inventory for the site was obtained from the West Virginia Environmental Quality Board (WVEQB), which provided API numbers for 25 wells shipping wastewaters to the facility. Between 2002 and 2014 the UIC well injected approximately 1.5 million barrels (equal to ~178 million liters) of wastewater from the 25 production wells located in West Virginia. Information about these production wells was obtained from the West Virginia Department of Environmental Protection (WVDEP), Office of Oil and Gas permit database (<http://tags.dep.wv.gov/oog/>) as detailed in Table S1. All of the wells are producing natural gas, with 15 wells producing coal bed methane, while four wells are producing from the Marcellus Shale. An additional 4 wells are characterized as horizontal gas wells producing from the Lower Huron shale formation (Lower Huron is WVDEP nomenclature, USGS usage is Huron Member of the Ohio Shale). We were unable to find records for the remaining two wells listed in the WVEQB document.

The Wolf Creek watershed encompasses 4430 ha with Wolf Creek flowing to the northeast and joining the New River ~10 km downstream from the facility (Figure S10). The New River is used for recreation and is a local drinking-water source. The headwaters of Wolf Creek flow through areas of past surface coal mining that have since been covered or reclaimed and are primarily residential or agricultural land.¹⁹ Despite reclamation, Wolf Creek was classified as biologically impaired by the WVDEP in 2008, due to high levels of iron and dissolved aluminum,²⁰ which may be due to acid mine drainage from the Summerlee coal mine.²¹

An initial reconnaissance-sampling trip conducted in September 2013 indicated potential impacts to the headwaters requiring additional investigation (Table 1, Figure 1). To further study impacts from the disposal facility, samples were collected from two locations within the Wolf Creek watershed: 1) a small tributary that runs through the disposal facility and 2) a separate drainage (referred to as “background drainage”), Site 2, with no potential impact from the disposal facility (Figure 1A, Table 1). The tributary begins upstream from the disposal facility and runs adjacent to the disposal well, then

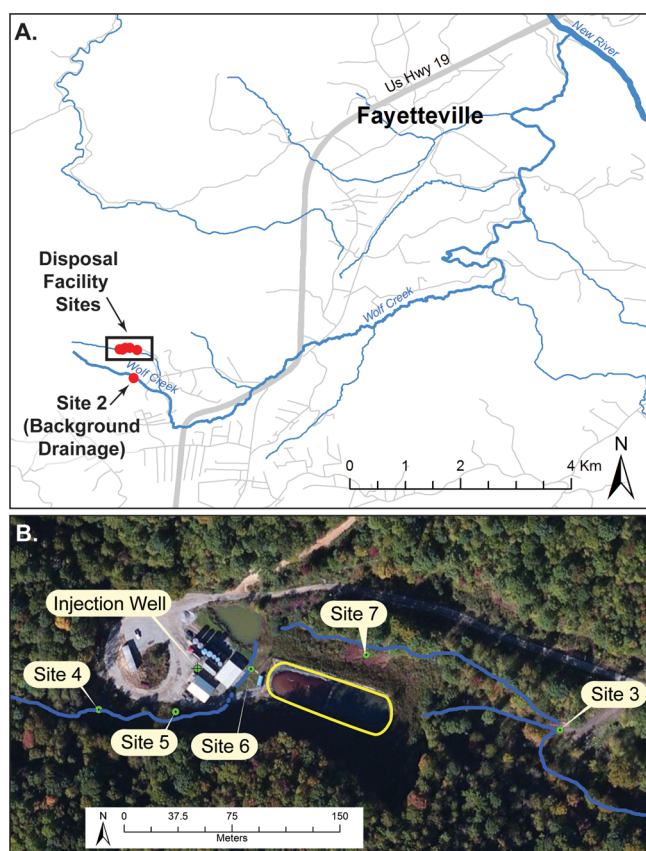


Figure 1. Map of sampling locations near Fayetteville, WV within the Wolf Creek watershed (A) and specific sites (B) in a stream running adjacent to a class II disposal facility. Panel A shows that Site 2 was located in a separate drainage from the disposal facility sites (outlined in black box), which are shown in panel B (Sites 4, 5, 6, 7 and 3). In panel B, the blue line highlights the stream, and the yellow outline is the location of the former impoundment ponds. Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community.

Table 1. Site Descriptions, Field Parameters and NVDOC of Water Samples Collected in September 2013, June 2014, and September 2014 in Tributaries of Wolf Creek^a

sample	type, location	pH			conductivity ($\mu\text{S}/\text{cm}$)			temp. ($^{\circ}\text{C}$)			NVDOC ^b (mg/L)		
		Sept. 2013	June 2014	Sept. 2014	Sept. 2013	June 2014	Sept. 2014	Sept. 2013	June 2014	Sept. 2014	Sept. 2013	June 2014	Sept. 2014
site 2	background, separate drainage	7.8	6.5	ND ^c	216	109	ND	14.1	16.0	ND	2.65	1.42	ND
site 4	background, upstream of disposal facility	ND	6.5	6.7	ND	74.0	125	ND	18.2	16.0	ND	1.13	1.60
site 5	adjacent to the disposal facility	ND	6.2	ND	ND	90.0	ND	ND	20.3	ND	ND	3.31	ND
site 6	adjacent to the injection well shed	ND	6.9	ND	ND	82.0	ND	ND	20.1	ND	ND	2.20	ND
site 7	adjacent to the former impoundment ponds	5.8	6.4	6.5	1750	416	1040	21.0	24.1	17.4	7.35	2.49	3.10
site 3	downstream of the disposal facility	6.4	6.1	6.5	1210	379	1110	25.0	22.9	17.7	3.85	3.24	3.40
field equipment blank	blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.86	0.50	0.70

^aSite locations are indicated in Figure 1. All field parameter values are the median of three individual measurements. Sites 7 and 3 were sampled in duplicate in June 2014 and values presented are averages. ^bNVDOC = non volatile dissolved organic carbon. ^cND = not determined.

downhill from the two former impoundments (Figure 1B). The background drainage-stream meets the disposal-facility-stream prior to joining the main stem of Wolf Creek, ~1000 m downstream from the facility (Figure 1A). No samples were obtained for the current study below Site 3 due to other industrial activities in the area (e.g., an automotive junkyard).

Site Sampling and Analysis. The disposal facility and background drainage streams (Site 2) were sampled in June 2014 at five points within the disposal facility, including locations upstream (Site 4), within (Site 5 and 6), and downstream (Sites 7 and 3) from the disposal facility (Figure 1B, Table 1). Conductivity, pH, and aqueous Fe^{2+} were determined in the field as described in the SI Methods. Water samples were collected from the approximate center of the stream for analysis of alkalinity, cations, anions, strontium (Sr), oxygen (O) and hydrogen (H) isotopes, nonvolatile dissolved organic carbon (NVDOC), trace inorganic elements, and disinfection byproducts (DBP) as described in the SI Methods. Additional field measurements and water samples for NVDOC and major and minor cations were collected in September 2013 and 2014 at Sites 2, 7, and 3. Streambed sediment samples were collected for carbon, nitrogen, and sulfur elemental analysis (CNS), Fe speciation, total inorganic elements, and microbial community analysis as described in the SI Methods. Detailed sampling, quality assurance/quality control (QA/QC) procedures, and analytical methods are described in the SI Methods. No hydrologic measurements were conducted or groundwater sampled during the September and June sampling efforts. Further investigations into the hydrology of the site were not possible due to site access limitations. The microbial community sequence data set was deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number SRP073303.

RESULTS AND DISCUSSION

Impacts to Stream Water Quality. Tributaries of Wolf Creek that were sampled in September 2013, June 2014, and September 2014 showed impacts on the tributary downstream from the injection disposal facility (Figure 1, Table 1). In September 2013 exploratory sampling indicated that there was elevated specific conductance at Sites 7 and 3 compared to the background drainage Site 2 (Table 1). Intensive sampling in June 2014 revealed that background sites located in the

separate drainage (Site 2) or upstream (Site 4), with no known UOG wastewater inputs, exhibited no visual signs of impacts or disturbance due to anthropogenic activity. Waters at these sites had neutral pH and specific conductance ($\sim 80 \mu\text{S}/\text{cm}$, Table 1) in line with that of minimally impacted streams in the area. Sites 5, 6, 7 and 3, which were located on the facility, adjacent to the disposal injection well, adjacent to the former holding ponds, or downstream, respectively, all exhibited visual signs of impacts. All 4 of these sites had red-orange sediments and waters characterized by neutral pH (Table 1). At Sites 6, 7, and 3 there were hydrocarbon odors once the sediment was disturbed, although Site 6 waters had a specific conductance in line with background sites and Site 5. Waters from sites downstream from the former impoundments (Sites 7 and 3) had elevated specific conductance ($\sim 400 \mu\text{S}/\text{cm}$) indicating that visual impacts at these sites were associated with alterations to the stream chemistry. The elevated specific conductance provided the field evidence that impacts to the stream are due to UOG wastewaters, because produced waters from both conventional and unconventional oil and gas wells in the Appalachian Basin are characterized by high salinity.^{5,6,10,22–26} Specific conductance at all of the sites was lowest at the June 2014 sampling time point, likely due to the fact that 3.0 cm of precipitation fell in 24 h on June 12, 2014 in nearby Beckley, WV,²⁷ less than 1 week prior to our sampling on June 17, 2014.

Water samples collected in June 2014 showed clear differences in chemistry upgradient and downgradient from the waste-disposal facility (Figures 2 and 3 and Table S2). Streams in this region are naturally low in NVDOC: below 1.5 mg/L at both background sites at the time of sampling (Figure 3a, Table S2). Concentrations increased to 2.2–3.3 mg/L at sites on and downgradient from the facility. In contrast, nitrate (NO_3^-) and sulfate (SO_4^{2-}) concentrations in the stream decreased on and below the disposal facility. Total Fe concentrations adjacent to the former impoundments were 8.1 mg/L, far in excess of the 0.13 mg/L found upstream from the facility (Table S2).

Chloride (Cl^- , 115 mg/L) and bromide (Br^- , 0.80 mg/L) concentrations were elevated adjacent to the former impoundments (Site 7), compared to 0.88 mg/L Cl^- and <0.03 mg/L Br^- at Site 4, upstream from the facility (Figure 3b,c and Table S2). The concentrations at Site 7 correspond to a Cl/Br ratio (mass basis) of ~ 144 , which is in-line with produced waters

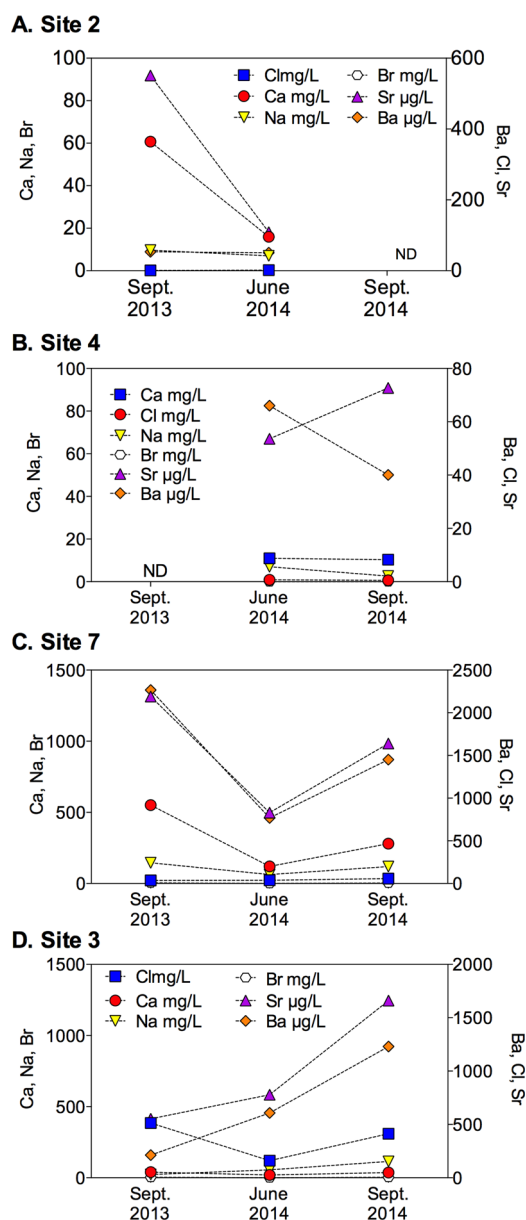


Figure 2. Major anion and cation concentrations over time at Sites 2 (A), 4 (B), 7 (C), and 3 (D). Samples were collected in September 2013, June 2014, and September 2014 at all sites with the exception of Sites 2 and 4, which were only sampled in Sept. 2013 and June 2014 and June and September 2014, respectively (ND = not determined). Note the different scales in panels A and B.

derived from evaporated paleoseawater.²⁸ Dissolved calcium (Ca^{2+}), sodium (Na^+), strontium (Sr^{2+}), and barium (Ba^{2+}) concentrations were also elevated at Sites 7 and 3 (Figure 3b,c). Due to the very low concentrations of Cl^- and Na^+ in the background water, these elements and Br^- , Sr^{2+} and Ba^{2+} , serve as a good local tracer of UOG wastewater impacts.²⁹ Iodine can be another indicator of UOG wastewater impacts;³⁰ however, concentrations were below the detection limit of 1 mg/L at all sites. Concentrations of several trace elements were also elevated above background, particularly lithium (Li^+), which is present at over 6 times the background concentration at Site 7 (Table S2, Figure 3e). Increased trace element concentrations in surface waters may be due to mobilization from sediments by the wastewater-derived inorganic salts, similar to what has been

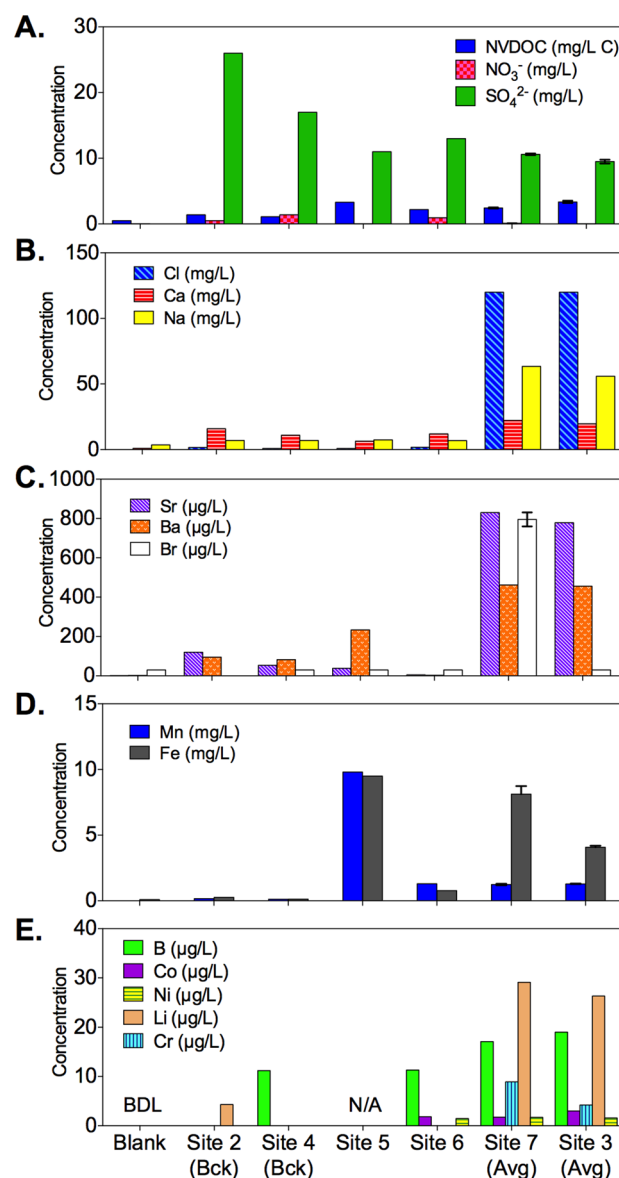


Figure 3. Chemistry in waters collected in June 2014 from a stream adjacent to the disposal facility. (A) Concentrations of nonvolatile dissolved organic carbon (NVDOC), sulfate, and nitrate; (B) chloride, calcium, and sodium concentrations; (C) strontium, barium, and bromide concentrations; (D) concentrations of dissolved Mn and Fe; and (E) trace element concentrations. Site locations are indicated in Figure 1 and Sites 2 and 4 are background (Bck) sampling locations. Site 2 is located in a separate drainage, whereas Site 4 is upstream of the disposal facility. Sites 7 and 3 were sampled in duplicate and averages (Avg) of individual measurements are presented. The blank is the field equipment blank.

observed for roadside soils impacted by deicing salts.^{31–39} Increased concentrations of Cl^- , Br^- , Sr^{2+} , and Ba^{2+} have been found in Pennsylvania streams downstream from wastewater treatment plants.^{30,40} Elevated Br^- concentrations in UOG wastewaters can lead to increases in disinfection byproducts (DBP) downstream from wastewater treatment plants,⁴¹ however DBP were not observed at any of the sites sampled in the Wolf Creek tributary.⁴²

Although the concentrations of Cl^- between Sites 7 and 3 did not change (indicating minimal dilution between the sites), concentrations of Ca^{2+} and Na^+ decreased by 12%, Ba^{2+} and Li^+

by 9% and Sr^{2+} by 6%. Bromide decreased to below detection at Site 3. The losses from solution of these elements indicate potential for incorporation into the stream-bed sediments. Skalak et al. 2014,⁴³ found the accumulation of Sr, Ca, and Na (in addition to Ra) in soils in areas of Pennsylvania where road spreading of produced-water brines was a common approach for deicing. Comparing the June 2014 results to those from September 2013 and 2014 shows that most elements that were elevated compared to background sites in June were lower in absolute concentration than observed during the September samplings (Figure 2), indicating dilution associated from recent rain events prior to the June 2014 sampling.²⁷

Isotopic Indicators of UOG Wastewater Sources.

Isotopic data for H, O, and Sr in samples collected in June 2014 show marked differences (Figure 4; Tables S2 and S5). Background samples from Sites 2 (background drainage) and 4 (upstream), exhibit lower Sr concentrations and higher $^{87}\text{Sr}/^{86}\text{Sr}$ (>0.713), than the samples on or below the disposal facility suggesting contribution from additional sources of water into the stream. Because Sr isotopes do not fractionate appreciably in the environment, sources of Sr-rich water with a lower $^{87}\text{Sr}/^{86}\text{Sr}$ ratio appear to contribute to the stream near Site 6 and again below the disposal facility (Sites 3 and 7). For context, these data are compared against late stage produced waters from the Marcellus Shale from Greene County, Pennsylvania and mine drainage water from the various Pennsylvanian age coals in the area (external Sr data from⁴⁴). There is some spatial variability in strontium isotope geochemistry across the Marcellus Shale, so data from the closest county (Greene) were used. On this type of plot ($^{87}\text{Sr}/^{86}\text{Sr}$ vs $1/\text{Sr}$), mixtures between any two end-members plot as straight lines (Figure 4a). The data point for the Site 6 sample falls along a mixing line between upstream water (Site 4) and Marcellus Shale produced waters (0.004%). Such small contributions can be identified because of the high concentration of Sr in Marcellus Shale produced water (>1500 mg/L) relative to the streamwater (<0.1 mg/L). The Sr signatures for samples from Sites 3 and 7, downstream from the disposal site are markedly different from the Site 6 sample. Their compositions overlap with data from Pittsburgh coal mine water, potentially suggesting an input of up to 50% of CBM produced waters in these samples. Loss of the apparent Sr signature from Marcellus Shale produced waters in these samples relative to the upstream Site 6 sample may be due to an overprinting by the relatively Sr-rich coal-sourced water (~ 1.2 mg/L coal-sourced water contribution vs ~ 0.1 mg/L contribution from the Marcellus Shale produced water).

Results from the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ analyses (Figure 4b) indicate that all of the samples collected are dominated by local meteoric water. Produced water samples from the Marcellus Shale from southwestern Pennsylvania are located distal to the local meteoric water line (LMWL) related to their origin from surface water mixing with formation water, which is highly evaporated paleoseawater.⁵ No published $\delta^{18}\text{O}$ and $\delta^2\text{H}$ data exist for conventional oil and gas wells produced waters in nearby areas, but examination in other parts of the basin show overlap between Marcellus Shale produced waters and those from conventional oil and gas wells.^{5,23} Thus, these data show no indication of mixing with substantial quantities of Marcellus Shale produced waters or likely any local conventional hydrocarbon produced waters. However, with a potential

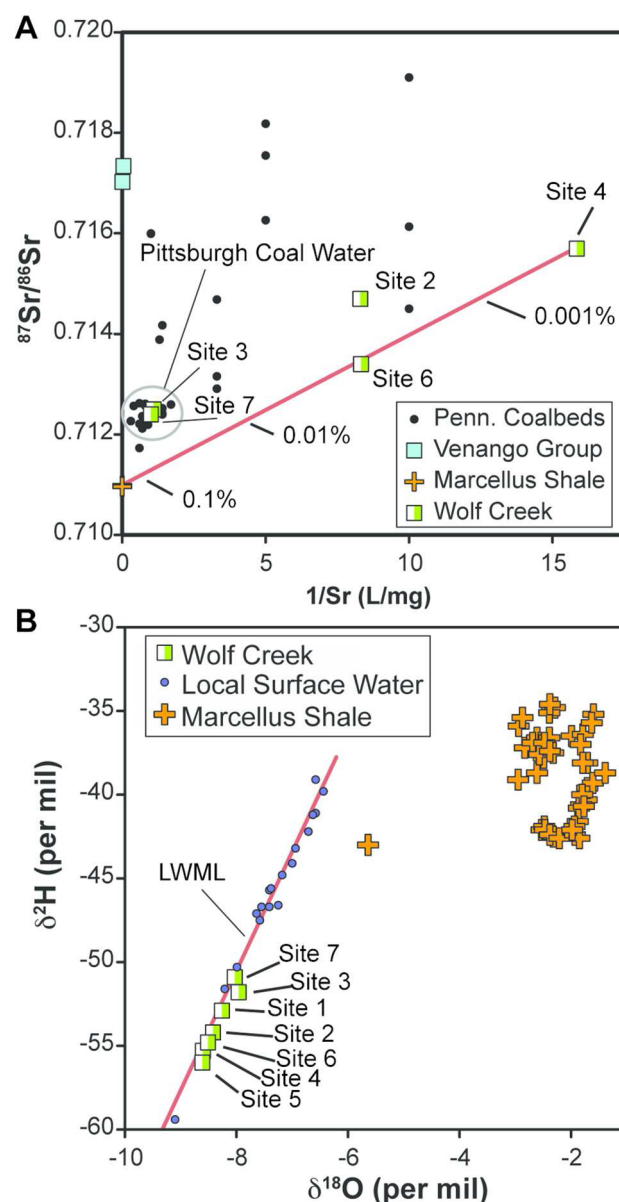


Figure 4. Two-component mixing plots of $^{87}\text{Sr}/^{86}\text{Sr}$ against $1/\text{Sr}$ concentration for Wolf Creek tributary water samples (green/white squares). Values for Pennsylvania coal beds (circles), Venango Group brines (blue squares), and Marcellus Shale produced waters (crosses) are provided for reference. Red line in panel A shows mixing pathway between Marcellus Shale produced water and Site 4. Percentages along the pathway indicate relative contribution of Marcellus Shale produced water in the mixture. Red line in panel B is the local meteoric water line (LMWL).

contribution of $<1\%$ such as possibly predicted from Sr isotopes at Site 6, no shift in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ would be expected. Estimated recharge temperatures based on equations by Dansgaard⁴⁵ are slightly warmer at the sites below the disposal facility (mean = 8.1 °C) than those for the remaining sites (mean = 7.4 °C), and their compositions cover the range of local surface waters (data from ref 46) suggesting a relatively shallow recharge source for the waters from Sites 3 and 7, such as coal mine adits or CBM water, although no local $\delta^{18}\text{O}$ and $\delta^2\text{H}$ data are available for either.

Characterization of Stream Sediments. Sediment samples for total elemental analysis and extractable iron

analyses were only obtained in June 2014. The percent carbon composition of the samples ranged from less than 1% to 5.4% with less than 1% of N and S. The C, N, and S contents of the sediments were consistent among sites with the exception of elevated C at site 6, which might be due to surface activities on site. The bulk sediment cation and trace element concentrations were similar between upstream, background and downstream streambed sediments (Figure S11 and Table S3), indicating no measurable impact from the waste disposal facility. Total Ca and Na concentrations observed were much lower (<2.5 mg/g; Table S3) than that observed in sediments impacted by oil and gas wastewaters (0.015–25 mg/g Ca and 0.01–48 mg/g Na;⁴³).

Mercury and uranium concentrations (Table S4) in sediment samples were within the range of values estimated for average upper crustal rocks⁴⁷ and showed no overall differences between downstream, impacted and background sites (Table S4 and Figure 5). In contrast, the ²²⁶Ra concentration at Site 6

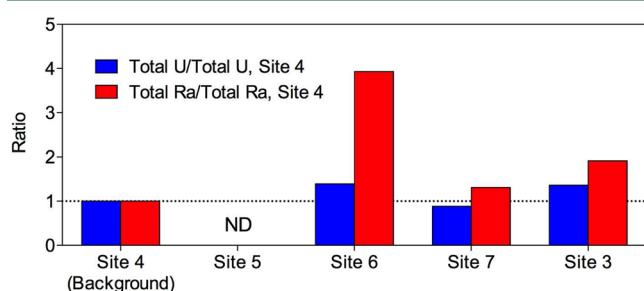


Figure 5. Ratios of total U and Ra in sediments collected from 5 sites along a stream adjacent to the disposal facility. Site locations are shown in Figure 1. Concentrations of total U and Ra are available in Table S4. No ²²⁸Ra was detected so Total Ra equals ²²⁶Ra.

was elevated well above background (²²⁸Ra concentrations were below detection in all samples). In contrast to all other sites, ²³⁸U/²²⁶Ra in sediments at Site 6 were not in secular equilibrium (Table S4) indicating an external source of ²²⁶Ra to the sediments. Elevated ²²⁶Ra activity, a product of ²³⁸U decay, is characteristic of produced water from the U-rich Marcellus Shale (e.g., ref 12), and elevated Ra concentrations were observed near a Marcellus Shale wastewater treatment facility discharge site⁴⁰ and in areas where conventional oil and gas wastewaters were used for road deicing.⁴³ The excess ²²⁶Ra detected in sediments at Site 6 is consistent with the Sr isotope data that suggest a small contribution of Marcellus Shale produced water in water samples from the same location. The ²²⁶Ra at Sites 3 and 7 appears to be in secular equilibrium with ²³⁸U and suggests negligible input of external ²²⁶Ra at these sites. This is consistent with input from coalbed methane produced waters, as they generally contain very little radium (<20 pCi/L).⁴⁸

Total iron concentrations were higher at Site 3 (Figure S11), but iron extractions showed that biogenic Fe(II) and bioavailable Fe(III) were elevated at Sites 7 and 3 (Table S4). Sites 7 and 3 had similar extractable Fe(III) concentrations, in agreement with the field observations of red-orange iron oxide-rich sediments. However, Site 7 duplicate field samples were highly variable visually (color, texture) and this is reflected in the variability seen in iron values between samples. One of the Site 7 samples was highly reduced, as shown by high Fe(II) contents (1340 μ mol/g sediment; Table S4) and low %

of Fe(III) and corresponded to a dark gray-black color of the sediments. The elevated iron contents at the site are likely associated with small-scale heterogeneities and potentially past coal mining in the area,^{20,21} but wastewater contamination may drive the distribution between biogenic Fe(II) and bioavailable Fe(III).

Microbiology. Analysis of Illumina sequence reads of the 16S rRNA gene v4 region revealed striking differences in microbial community structure in the streambed sediments upstream and downstream of the disposal facility (Figure 6, Table S8). The alpha diversity was observed to be much lower at Site 7 (Inverse Simpson Index of 377), adjacent to the former impoundments, than either downstream at Site 3

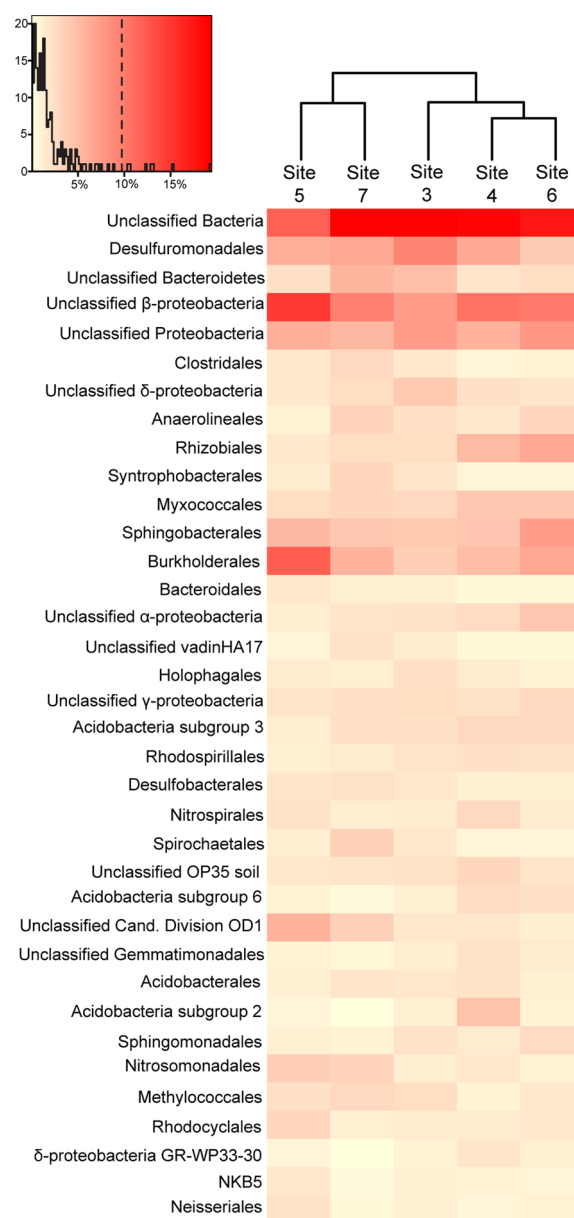


Figure 6. Heatmap and dendrogram of microbial orders comprising >1% of microbial communities in sediments collected from 5 sites along a stream adjacent to the disposal facility in June 2014. Site locations are indicated in Figure 1, and sediments were collected from the upper 5 cm of the streambed. Dendrogram represents relatedness of communities between sites.

(Inverse Simpson Index of 1063) or upstream at Sites 4 and 6 (Inverse Simpson's Indices of 689 and 787, respectively). A heatmap was constructed to visualize differences in the structure of the microbial community using bacterial orders of greater than 1% abundance combined with cluster analysis (Figure 6). Notably, anaerobic orders including the Desulfurimonadales, Anaerolineales, and Syntrophobacterales were found at greater abundance at Sites 7 and 3, suggesting anaerobic conditions in the shallow streambed.^{49–51} In addition, Clostridiales were observed in greater abundance at Sites 7 and 3, a finding similar to that in other UOG wastewater influenced systems.^{6,52} In contrast, the predominantly aerobic Rhizobiales,⁵³ Myxococcales,⁵³ and Sphingobacteriales⁵⁴ were found in greater abundance at Sites 4 and 6.

Canonical correspondence analysis⁵⁵ and the *ENVEFIT* function in the *R* vegan⁵⁶ package were utilized to relate differences in microbial community structure to the measured geochemical parameters of the streamwater (Figure S12). The community at Site 7 separates from Site 4 along axes 1 and 2, while the community at Site 3 separates from Site 4 along axes 1 and 3. In this analysis, the elevated dissolved metal concentrations observed at Sites 7 and 3 vary along axes 1 and 2, suggesting a relationship between the elevated metals found at these sites and the shifts in microbial community composition. Eigenvectors and loadings for Figure S12 are presented in Table S9.

Reactions That Control Element Stability/Fate and Transport. TCO_2 (total dissolved carbon dioxide) concentrations and P_{CO_2} (partial pressure of carbon dioxide) values were calculated from the solution compositions (Table S7). P_{CO_2} values at all sites ranged from 1.8% to 5.7%, substantially higher the atmospheric value of 0.04%, indicating the impact of respiration on the streamwater chemistry. Calcite, rhodocrosite (MnCO_3), and siderite (FeCO_3) were undersaturated but approached saturation with respect to rhodocrosite at Sites 5 and 6 and siderite at Sites 5 and 7. Saturation with respect to barite (BaSO_4), which can control both Ba^{2+} and Ra^{2+} concentrations and act as a reservoir for these elements in sediments,⁵⁷ was examined using the streamwater chemistry. Barite was undersaturated or slightly supersaturated at all sites, including the background sites (Table S7). Maximum saturation indices were observed at Site 7 in September 2014 (0.7) and Site 3 in June 2014 (0.2–0.5). Barite precipitation at these low degrees of saturation is unlikely given the inhibition by humic and fulvic acids, principal constituents of NVDOC, at NVDOC concentrations observed during this study (Table S2).⁵⁸ Thus, Sr^{2+} , Ba^{2+} , Ra^{2+} , and other metal ions elevated owing to UOG wastewater impacts are likely present as sorbed species in the sediments rather than incorporated into minerals and, as such, could be mobilized upon changing chemical conditions.

Implications. Multiple lines of evidence demonstrate that activities at the disposal facility are impacting the stream that runs through the area, as shown by changes in the inorganic chemistry and microbiology at the downstream sites. In addition, collaborative papers examining the organic chemistry and endocrine disrupting activity⁵⁹ in the same samples, provide additional lines of evidence demonstrating that activities at the disposal facility are impacting the nearby ecosystem. Many of the inorganic constituents known to be associated with UOG wastewaters and Appalachian Basin brines, e.g., Cl^- , Ca^{2+} , Na^+ , Sr^{2+} , and Ba^{2+} ,^{5,6,10,22–26,29,30} were elevated in streamwater samples downstream of the disposal facility, indicating that the impacts were associated with UOG

wastewater inputs. Indeed, Site 7 waters had Cl^- and Br^- concentrations consistent with the influence of wastewater brines from conventional and unconventional resources, with concentrations consistent with coal bed methane,²⁵ Marcellus Shale produced waters,^{9,30} and produced waters from Appalachian Basin conventional oil and gas wells.^{23,30} However, the contribution of wastewaters to the stream chemistry is small, but still detectable, with less than 0.001 part brine to 0.999 parts freshwater needed to account for the observed stream Br^- and Cl^- contents.

Inorganic components of brine can immediately impact water quality, and can potentially alter ecosystem functions by impacting biogeochemical nutrient cycling. For example, increases in salinity due to deicing of roads are associated with disruptions in nitrogen cycling, likely due to alterations of microbial communities.^{60,61} The alterations in sediment microbial communities at the downstream sites could impact nutrient cycling in the stream, highlighting the importance of understanding the link between microbial community structure and function in environments impacted by UOG wastewater releases. Increasing hardness and metal concentrations in ecosystems impacted by road salt were also shown to have toxic effects on aquatic organisms and terrestrial plants.^{38,62,63} At Wolf Creek, organisms may be similarly impacted; similar components are elevated in stream waters due to disposal activities and wastewater inputs.

Our findings show that the disposal facility is impacting the stream but we are unable to identify a point source of contaminants to the stream. Disposal facilities offer multiple potential sources, including leaking wastewaters from storage ponds and tanks, as well as from fuel and motor oil from vehicles making frequent deliveries (e.g., wastewater transport). Contaminants from impoundment ponds or spills can reach streams by overland flow or through groundwater discharge that leach into the subsurface through failed or incomplete liners. In addition, background concentrations in streams may be elevated owing to previous land use, such as coal mining, which highlights the necessity of identifying and sampling an appropriate background site (e.g., upstream). Further investigations of potential contaminants, endocrine disruption activity of stream waters, as well as studies of aquatic organisms, and comparisons with impacts from other anthropogenic inputs are warranted to determine potential environmental health impacts of UOG wastewater disposal practices.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b00428.

Detailed descriptions of site sampling, quality assurance/quality control (QA/QC) procedures and analytical methods; supporting references; Tables S1–S9 and Figures S10–S12 (PDF)

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Notes

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The authors declare no competing financial interest.

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Environmental signatures and effects of an oil and gas wastewater spill in the Williston Basin, North Dakota

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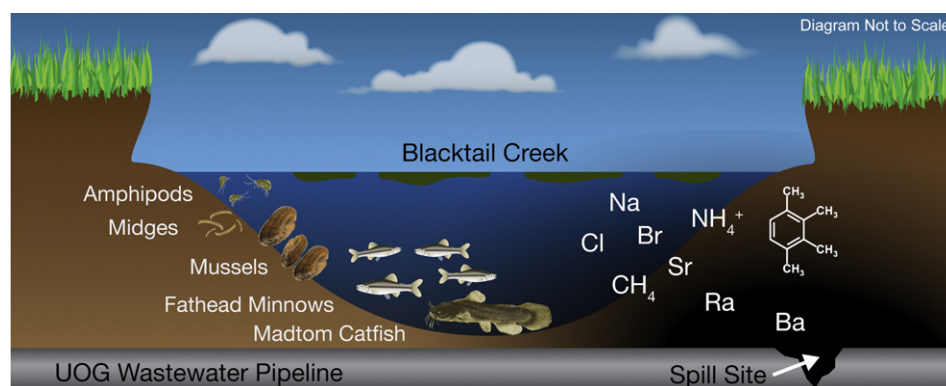
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HIGHLIGHTS

- UOG wastewater (>11 million liters) spilled into Blacktail Creek, ND in January 2015.
- Elevated Na, Cl, Br, Sr, B, Li, NH₄, and hydrocarbons were detected in creek waters.
- Geochemical baseline deviations persist months after remediation efforts started.
- B and Sr concentrations, and Ra activities were up to 15 times background in sediment downstream.
- Biological impacts include reduced fish survival and estrogenic inhibition downstream.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewaters from oil and gas development pose largely unknown risks to environmental resources. In January 2015, 11.4 M L (million liters) of wastewater (300 g/L TDS) from oil production in the Williston Basin was reported to have leaked from a pipeline, spilling into Blacktail Creek, North Dakota. Geochemical and biological samples were collected in February and June 2015 to identify geochemical signatures of spilled wastewaters as well as biological responses along a 44-km river reach. February water samples had elevated chloride (1030 mg/L) and bromide (7.8 mg/L) downstream from the spill, compared to upstream levels (11 mg/L and <0.4 mg/L, respectively). Lithium (0.25 mg/L), boron (1.75 mg/L) and strontium (7.1 mg/L) were present downstream at 5–10 times upstream concentrations. Light hydrocarbon measurements indicated a persistent thermogenic source of methane in the stream. Semi-volatile hydrocarbons indicative of oil were not detected in filtered samples but low levels, including tetramethylbenzenes and di-methylnaphthalenes, were detected in unfiltered water samples downstream from the spill. Labile sediment-bound barium and strontium concentrations (June 2015)

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Endocrine disrupting activity

were higher downstream from the Spill Site. Radium activities in sediment downstream from the Spill Site were up to 15 times the upstream activities and, combined with Sr isotope ratios, suggest contributions from the pipeline fluid and support the conclusion that elevated concentrations in Blacktail Creek water are from the leaking pipeline. Results from June 2015 demonstrate the persistence of wastewater effects in Blacktail Creek several months after remediation efforts started. Aquatic health effects were observed in June 2015; fish bioassays showed only 2.5% survival at 7.1 km downstream from the spill compared to 89% at the upstream reference site. Additional potential biological impacts were indicated by estrogenic inhibition in downstream waters. Our findings demonstrate that environmental signatures from wastewater spills are persistent and create the potential for long-term environmental health effects.

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1. Introduction

Liquid and solid wastes produced during development of oil and gas resources, particularly those from unconventional oil and gas (UOG) resources that are produced using horizontal drilling and hydraulic fracturing technologies, pose potential, but largely unquantified, risks to environmental resources and the health of organisms, including humans. Although several site investigations have been conducted, (e.g., Akob et al., 2016; Lauer et al., 2016; Rivard et al., 2014; Skalak et al., 2014; Warner et al., 2014), the significant variation in the composition of produced water (defined in Engle et al., 2014, referred to as UOG wastewaters herein) from shale and tight oil formations, (e.g., Akob et al., 2016; Blondes et al., 2014; Engle et al., 2016; Harkness et al., 2015; Rowan et al., 2015), as well as differences in disposal methods, climate, and physiography, have limited our understanding of environmental effects of UOG wastes. Recent extensive increases in hydrocarbon production from the tight oil reservoirs in the Williston Basin, including the Bakken and Three Forks Formations, have resulted in increases in water use (Gallegos and Varela, 2015; Scanlon et al., 2016; Scanlon et al., 2014) and new sources and increased volumes of wastewaters (Engle et al., 2014; Gallegos and Varela, 2015; Horner et al., 2016; Kondash and Vengosh, 2015).

Recent studies have raised concerns about the environmental and human health impacts of UOG wastes and have underscored the substantial knowledge gaps in understanding the ecological and human health hazards associated with waste releases (Elliott et al., 2016; Kassotis et al., 2016a; Kassotis et al., 2016b; Vengosh et al., 2014; Yost et al., 2016). Knowledge gaps include identifying pathways to exposure and the chemicals most likely to pose a hazard (Rogers et al., 2015). Tracking UOG wastewaters through their life-cycle of use has required development of new analytical approaches and modeling tools. Recent studies have shown that $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, for example, are useful geochemical fingerprints of wastewaters because trapped formation waters can develop distinct isotopic signatures, which are conservative and contain high concentrations of Sr (Lauer et al., 2016; Peterman et al., 2012; Stewart et al., 2015; Warner et al., 2013). Further development of analytical methods for trace levels of organics, stable and radioactive isotopes, and compounds of environmental health concern, including endocrine disruptors, are critical to a better understanding of the impact of releases.

Wastewaters from UOG production can be highly saline (often referred to as brine) and contain a complex mixture of chemicals, including toxic and radioactive elements from the formation and chemical additives from production (Barbot et al., 2013; Engle et al., 2014; Gregory et al., 2011), which may enter the environment in a variety of ways including direct disposal or well-stimulation practices (DiGiulio and Jackson, 2016; Skalak et al., 2014; Warner et al., 2014), accidental leaks or spills (Akob et al., 2016; Drollette et al., 2015) or beneficial uses such as de-icing on roadways or agricultural irrigation (Adams, 2011; Engle et al., 2014; Lutz et al., 2013; Skalak et al., 2014). Surface releases of wastewaters have been hypothesized to be the source of trace organic compounds in residential wells in the Marcellus Shale region of Pennsylvania (Drollette et al., 2015). Activities at a wastewater disposal

facility in West Virginia caused changes in stream chemistry and microbiology, and measurable endocrine disrupting potential (Akob et al., 2016; Kassotis et al., 2016a). Inorganic contaminants from brine spills in North Dakota have been found to persist at elevated levels in surface water from months to years (Lauer et al., 2016). In addition to the work conducted by Lauer et al. (2016), two previous studies in the Bakken region on the effects of UOG wastewater in the environment found evidence of brine contamination both in surface and groundwater at a total of four different study site locations (Gleason and Tangen, 2014; Mills et al., 2011). These studies highlight the need to determine both the long-term effects and a more comprehensive understanding of the constituents released into the environment from UOG activities. It has become increasingly clear that management of wastewaters from UOG extraction is a key issue in avoiding environmental damage (Lutz et al., 2013; Vidic et al., 2013).

In North Dakota, the boom in tight oil production ramped up in 2008 and has been associated with an increase in the number of spills at production and disposal sites. An analysis of spills data reports from 2008 to 2015 (detailed methodology presented in the SI Methods) available from the North Dakota Department of Health, revealed that >8000 spills of fluids were recorded (Fig. 1). Despite the fact that volumes and compositions of materials spilled are often not reported, almost 53 million liters of “brine” spills were documented in the reports, representing the vast majority of spills by volume. The documented spills constituted over 75 million liters of total fluids, 17 million liters of which was classified as oil. Most of the spills have occurred in a relatively few number of watersheds in the northwest corner of the state (Fig. 1). This has led to some watersheds (examined at the HUC 10 scale, (Seaber et al., 1987)) cumulatively receiving over 2.5 M L of reported spills and this is likely a conservative estimate. Lauer et al. (2016) demonstrated an association between oil well density and incidents of wastewater (brine) spills in North Dakota.

In January 2015, a UOG wastewater pipeline leak was reported in northwestern North Dakota that allowed an estimated 11.4 million liters of wastewater to flow into nearby Blacktail Creek. Blacktail Creek is a small tributary of the Little Muddy River, which flows into the Missouri River (Fig. 2). We are using the large magnitude spill into Blacktail Creek to conduct in-depth interdisciplinary studies of the environmental fate and potential health effects of contaminants from UOG wastewater releases that enter a surface-water body. This study aimed to identify wastewater constituents that entered the environment, to understand how these components partitioned between water and sediment in the fluvial environment, and to evaluate some of the potential impacts to ecological or human receptors. Samples collected during two time periods, February and June 2015, indicate the presence of wastewater markers and biological impacts in the river; these results demonstrate the persistence of geochemical alterations six months after the spill was discovered. Partitioning of elements from the wastewater spill onto sediment attenuates contaminant concentrations and decreases rates of aqueous transport but could provide a long-term source which could negatively affect the aquatic ecosystems. This work builds on the initial observations of Lauer et al. (2016), who

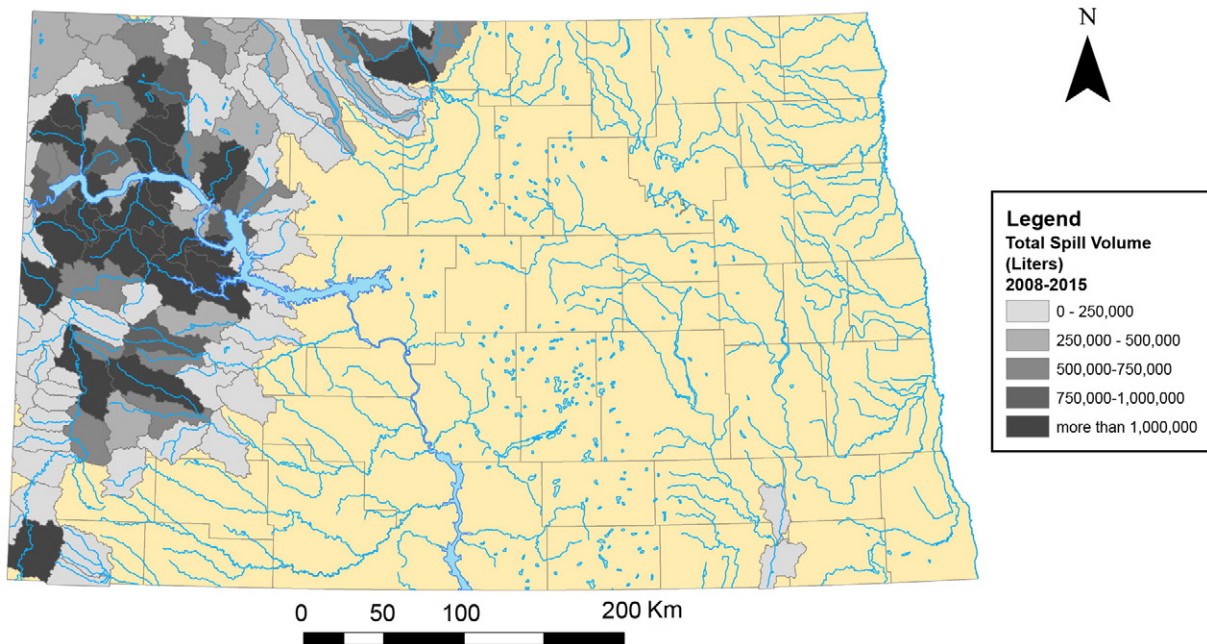


Fig. 1. Cumulative spills from oil and gas production on watersheds in North Dakota between January 2008 and June 2015. Watershed volumes were calculated by combining reported values of oil, brine, and unidentified liquid waste accidentally released during drilling operations.

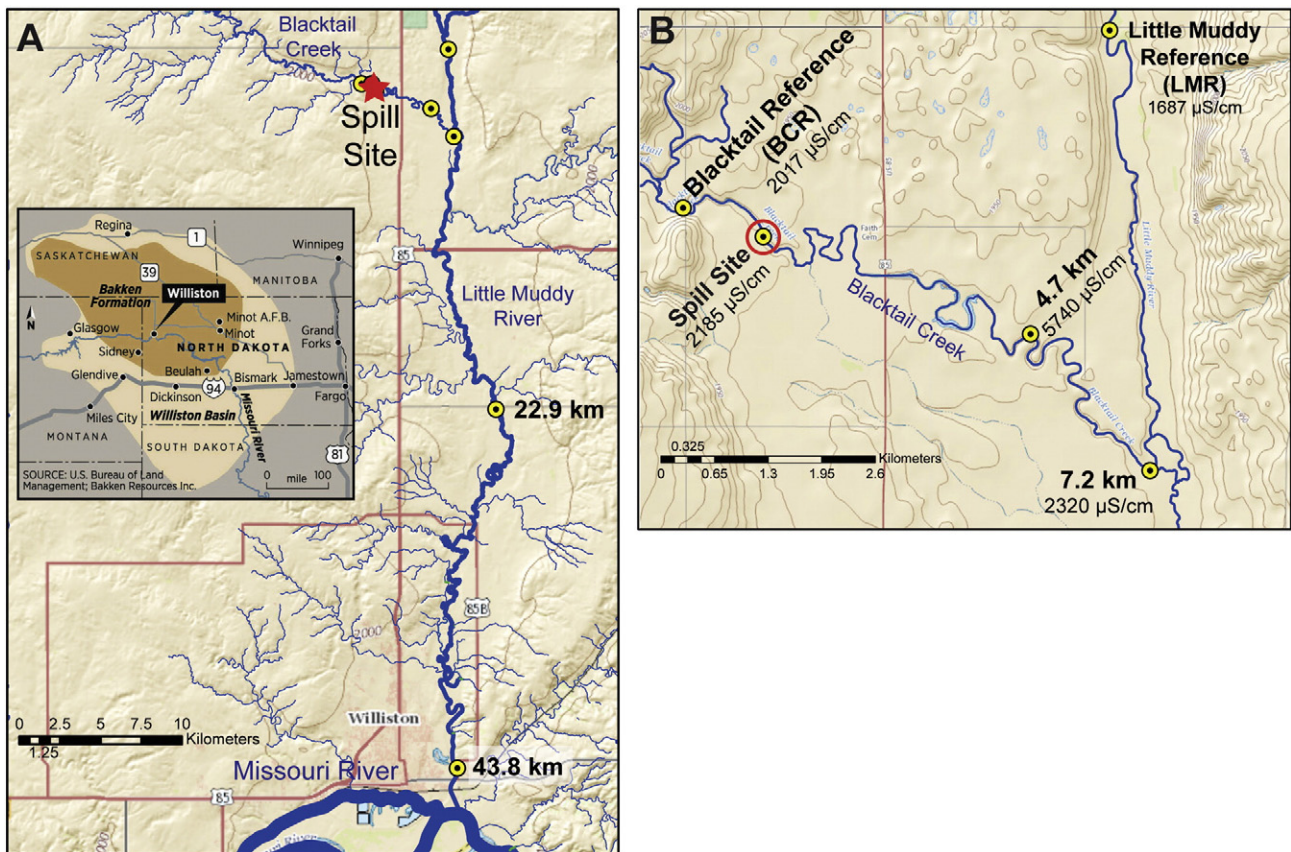


Fig. 2. Map of sampling locations along Blacktail Creek and Little Muddy River (A) tributaries of the Missouri River. Panel (B) shows specific sites located along Blacktail Creek and proximity to the area of the spill (red circle). Specific conductance values were those measured in February 2015 (Table S9). Inset in (A) shows location within North Dakota. Map data from: USGS The National Map: National Boundaries Dataset, National Elevation Dataset, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; U.S. Census Bureau – TIGER/Line; HERE Road Data.

collected and analyzed two samples of stream water from the study site in July 2015 and concluded, assuming a contribution of produced water from the Bakken, that the pipeline fluid had entered the creek.

2. Materials and methods

2.1. Site description

The study area is located along Blacktail Creek and the Little Muddy River just north of Williston, North Dakota (ND, Fig. 2a). A leak, reported on January 6, 2015, from a shallow underground pipeline that crosses Blacktail Creek resulted in wastewaters from oil production in the Williston Basin to quickly enter into the stream. The leak was approximately 70 m from the stream on the west side of Blacktail Creek; the creek flows approximately 11 km before it joins the Little Muddy River and another 60 km until it flows into the Missouri River. Remediation efforts began in January 2015 and continue at the pipeline leak site, significantly altering the natural hydrology and disturbing much of the surrounding area. Satellite imagery and field reconnaissance personnel indicated that approximately 10,000 m² of surface soil and sediment has been removed at the site to a depth of 10–30 cm. The stream has been temporarily impounded at several locations by low headwater dams to collect and skim contaminated water. The groundwater between the Spill Site and 4 km downstream was pumped extensively during remediation in the spring and summer of 2015 to the extent that the stream was largely non-flowing in this region during our June 2015 sampling.

Blacktail Creek is a 3rd order tributary and its watershed is approximately 120 km² in size. Blacktail Creek joins the Little Muddy whose watershed is 1961 km² and it enters the Missouri River at Lake Sakakawea (formed by the Garrison Dam). Land use in the watershed is 72% agriculture and 4% residential, with the remaining 24% undeveloped (scrub/wetland) (Homer et al., 2015). Analysis of spills reports revealed that within the watershed (at HUC 10 level, Fig. 1), 200,014 L of brine, 35,246 L of oil, and 10,819 L of “other” liquid from 50 other reported spills from 76 new wells had been reported in the previous 7 years. A U.S. Geological Survey stream gage (USGS Site ID 06331000) located on Little Muddy, approximately 22.9 km downstream from the Spill Site, has continuous discharge data since 1954. Average annual discharge for Little Muddy River is approximately 0.92 cubic meters per second (cms) (<http://waterdata.usgs.gov/nd/nwis/>). Flow observations were collected at a temporary stream gage on Blacktail Creek near Marmon, ND (USGS Site ID 06330515) from March to November 2015 located 2.6 km downstream from the Spill Site. Remediation efforts have greatly affected the hydrology of the creek through the use of dams and groundwater pumping. The average daily discharge from March to November 2015 ranged from 0 to 3.39 cms with an average daily discharge of 0.15 cms. During the remediation period, Blacktail Creek had 129 days of zero flow out of the 256 days that the site recorded data. Significant flow variations in the stream occur longitudinally likely due to groundwater flow through observed paleochannels of coarser material along the bank.

2.2. Site sampling and analysis

Samples of sediment and water were collected on February 9–13 and June 15–28, 2015 at 7 sites along Blacktail Creek and the Little Muddy River (Fig. 2). Four of the sampling sites were located on Blacktail Creek: approximately 0.9 km upstream from the spill (representing background conditions for reference, site “BCR”), 0.1 km downstream from the spill (inside the area being remediated, “Spill Site”), 4.7 km downstream from the spill (just outside the area of remediation, site “4.7 km”), and 7.2 km downstream from the spill near the confluence of the Little Muddy River (site “7.2 km”) (Fig. 2b). The three sites on the Little Muddy River were located 4.5 km upstream from the confluence with Blacktail Creek (site “LMR”, representing the background

(reference) condition on the Little Muddy), at the USGS stream gage (site ID 06331000) located 22.9 km from the spill (site “22.9 km”), and near the confluence of the Little Muddy and the Missouri river 43.8 km from the spill in the town of Williston, ND (site “43.8 km”). Site locations were chosen to avoid the active remediation in progress and to focus on examining the downstream impacts. However, the Spill Site did experience active remediation efforts which likely affected the samples we collected there.

Duplicate samples were collected at two sites (7.2 km and at 22.9 km indicated by (1) and (2) in figures and tables). Fish bioassay studies were conducted at sites BCR, LMR, the Spill Site, and 4.7 km, 7.2 km, and 22.9 km downstream from the spill. Water samples for total dissolved ammonium (NH₄) analyses were collected after completion of the fish bioassay studies on June 26, 2015. Total dissolved NH₄ refers to the sum of all dissolved ammonium species, including NH₄⁺ and aqueous NH₃.

In February 2015, the surfaces of Blacktail Creek and the Little Muddy River were frozen and water samples were obtained by first augering through the ice layer (approximately 30–50 cm) as described in the Supplemental Information (SI) Methods and shown in Fig. S1. In June 2015, all filtration was performed on site, using in-line filtration. Detailed methods are described in the SI Methods. Field measurements included specific conductance, pH, and dissolved oxygen (DO). Water samples were collected and analyzed for alkalinity, cations, anions, ammonium, strontium (⁸⁷Sr/⁸⁶Sr) isotopes, trace inorganics, nonvolatile dissolved organic carbon (NVDOC), semi-volatile hydrocarbons and UOG production organic additives, low-level light hydrocarbons, extractable hydrocarbons, low molecular weight organic acids (LMWOA), and endocrine disrupting activities as described in the SI Methods. Sediment was collected from the upper 1 to 2 cm of the streambed and analyzed for carbon, hydrogen, nitrogen, sulfur (CHNS); barium, strontium, calcium, radium; extractable hydrocarbons; and for invertebrate bioassays. An unfiltered, acidified (HNO₃) sample of the pipeline wastewater was supplied by The North Dakota Department of Health and analyzed for NVDOC, ammonium, ⁸⁷Sr/⁸⁶Sr isotopes, cations, and anions using the methods described in the SI Methods. Geochemical model computations were conducted using the pipeline and surface water samples to assess saturation indices for key solid and gas phases as described in the SI Methods. Ninety-six hour in situ bioassays were conducted during June 17–21, 2015, with Fathead Minnows (*Pimephales promelas*, FHM) as described in the SI Methods. Survival was recorded every 24 h and field parameters (water temperature, DO, and specific conductance) were recorded hourly. Water samples for cation and anion analyses were collected daily during the in situ bioassays to assess total major ion concentrations.

3. Results and discussion

Historical discrete specific conductance values at the Little Muddy River stream gage (Fig. S2) retrieved on June 6, 2016 showed a mean specific conductance of 1858 µS/cm and a median specific conductance of 2060 µS/cm. Specific conductance in October 2014 and January 2015 exceeded 3000 µS/cm, representing the two highest recorded values since 1975, presumably reflecting the pipeline break upstream and spillage of wastewater with high total dissolved solids (TDS) into the river. Data collected for the current study, closer to the pipeline break, showed specific conductance values increased by approximately a factor of two 4.7 km downstream from the spill compared with upstream at BCR in February 2015 (Fig. 2b). Typical specific conductance values for streams in western North Dakota range from about 700 to 3300 µS/cm (Galloway et al., 2012).

3.1. NVDOC and major inorganic chemistry

The pipeline wastewater had a TDS concentration of at least 300 g/L, which is near the maximum TDS found in produced waters in the

United States but near the median for those from the Bakken Formation (250 g/L) (Blondes et al., 2014) (Table S7). Ammonium was the third most abundant cation (Table S7, S8), assuming near-neutral pH values in the pipeline fluid. The high NH_4 concentration determined in the spilled fluid sample is consistent with high concentrations of NH_4 in produced water samples from this region as reported elsewhere (Blondes et al., 2014; Lauer et al., 2016). Thus, the spill contributed a large mass (2 million mol, which equals 28 metric tons as N) of labile nitrogen to Blacktail Creek. As is evident from the data compiled in Table S7, concentrations of most solutes are close to their corresponding median values for produced waters from the Bakken Formation compiled in the USGS Produced Water Database (Blondes et al., 2014). One exception is sulfate (SO_4), whose concentration of 2900 mg SO_4 /L is over 6 times the median sulfate concentration in the database but less than the maximum concentration of 11,000 mg SO_4 /L. The exact reservoir(s) the pipeline fluid was sourced from is not known. Lauer et al. (2016) showed similarities between the composition of two Blacktail Creek samples from this site and produced waters from the Bakken but did not examine other potential sources. During the interval 2013–2015, ~40% of the new wells in the Bakken-Three Forks petroleum system were tight oil wells drilled into the Three Forks Formation (IHS Markit, 2016). There are no known publicly available produced water compositional or isotopic data for the Three Forks Formation. Thus, these discrepancies may be due to all or some of the water being sourced from non-Bakken reservoirs. Geochemical model computations conducted using the composition given in Table S7, a temperature of 25 °C, and model parameters described in the SI, show that the pipeline fluid composition is slightly supersaturated with halite and supersaturated with respect to celestite (SrSO_4), barite (BaSO_4), and both anhydrite and gypsum (at equilibrium with each other at this composition).

Fig. 3 shows the concentrations of major chemical components, NVDOC, chloride (Cl) and sodium (Na) in the pipeline sample and surface water samples. The concentration of NVDOC in the pipeline sample was 34 mg/L carbon (C). The upstream sample from Blacktail Creek (BCR) had NVDOC concentrations of 22 mg C/L and 21 mg C/L in

February and June, respectively. The LMWOA analyses showed low levels of lactate (0.3–0.5 mg/L) and trace amounts of formate (equal to and <0.1 mg/L) in all samples along Blacktail Creek (Table S9C). Because of the high amount of carbon naturally present in the creek, NVDOC is not a useful indicator of the presence of the wastewater in the surface water.

Chloride and Na are present in the pipeline brine at concentrations that are orders of magnitude higher than naturally present in either Blacktail Creek or Little Muddy River (Fig. 3). The background waters contain Cl concentrations below 15 mg/L and Na concentrations between 200 and 400 mg/L in both February and June 2015. At the Spill Site, concentrations of pipeline indicators were low (Fig. 3), due to the active damming and pumping of water at the site. Consequently, the chemistry at the Spill Site was similar to upstream water chemistry at BCR. However, at 4.7 km and 7.2 km downstream from the spill, Cl and Na were substantially elevated, with Cl present at 67 and 72 times the background concentration at BCR in February, respectively. These observations indicate that despite remediation activities, the downstream waters are impacted by the spilled wastewater. Molar Na/Cl ratios of excess Na and Cl (i.e., background-subtracted) in water samples from the 4.7 km site (0.83) from February matches molar Na/Cl ratios for typical Bakken produced water and the pipeline water sample (0.8–0.9), similar to the observations of Lauer et al. (2016) supporting the argument that produced water from the pipeline was the source of the increased salinity during this event. The pipeline Na/Cl signature will be preserved in water downstream from the spill if no more than a small fraction of the large mass of Na derived from the pipeline spill sorbed to stream sediments. Elevated Cl and Na concentrations were observed at sampling locations as far downstream as 22.9 km on the Little Muddy River. In June 2016 the concentration of Cl at 4.7 km and 7.2 km were 18 and 11 times higher than the concentrations at the upstream BCR site, respectively. However, in June 2015, the Cl signature of the spill was no longer detectable at the gage location 22.9 km downstream, indicating dilution of the wastewater signature. Complete chemistry for all samples is available in Table S9.

Using a simple mass balance mixing model for Cl between the BCR and pipeline data (as described in the SI Methods), we estimate that the pipeline fluid contributed 0.6% of Cl at 4.7 km and 0.44–0.45% of Cl at 7.2 km in February 2015 and 0.14% of Cl at 4.7 km and 0.08% of Cl at 7.2 km in June 2015 (Table S10). Almost no contribution of Cl from the pipeline fluid is predicted from this model at the Spill Site supporting the argument that remediation activities limited movement of pipeline fluid into Blacktail Creek near the Spill Site at the location and time of sampling.

The pipeline sample contained 825 mg/L bromide (Br), whereas Br concentrations were below detection in BCR upstream from the spill and in the LMR reference site (Fig. 4, Table S8 and S9). As was observed for Na and Cl, Br concentrations at the Spill Site reflect the chemistry of upstream water due to active remediation near the Spill Site. Elevated Br concentrations downstream from the spill were detected at 4.7 km and 7.2 km in February 2015, but not at sites farther downstream, where substantial dilution by the higher flows of low-Br water in the Little Muddy River attenuates Br concentrations. Bromide could still be detected at sites along Blacktail Creek downstream from the Spill Site in June 2015 (Fig. 4, Table S9). Other elements that were substantially elevated above background concentrations included Sr, lithium (Li) and boron (B) (Fig. 4). Boron was still detected at concentrations substantially above background at the gage, 22.9 km downstream. This observation is consistent with the findings of Lauer et al. (2016); in their study of a broad sampling of surface waters in ND affected by brine spills, including 2 samples from this study area collected in July 2015, they found that Br, Sr, Li, and B were positively correlated with Cl indicating they may behave conservatively. Many of these same elements were detected in surface waters downstream from a UOG wastewater injection facility in West Virginia (Akob et al.,

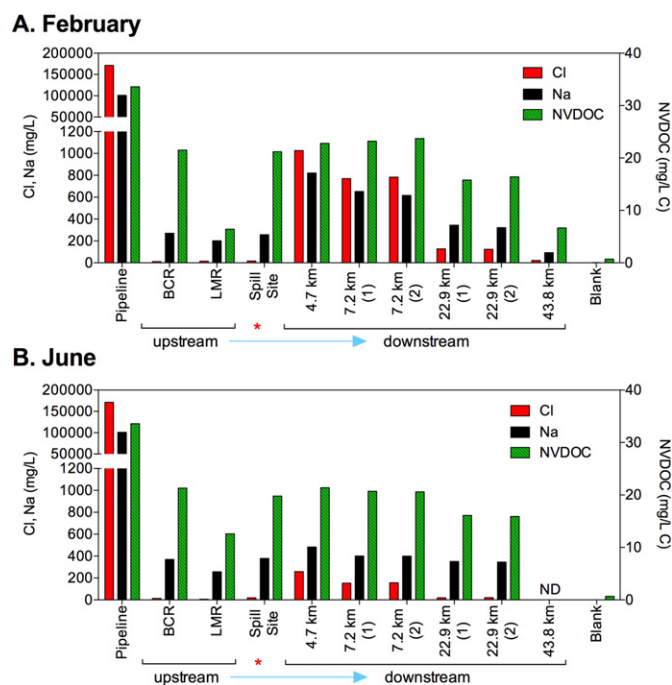
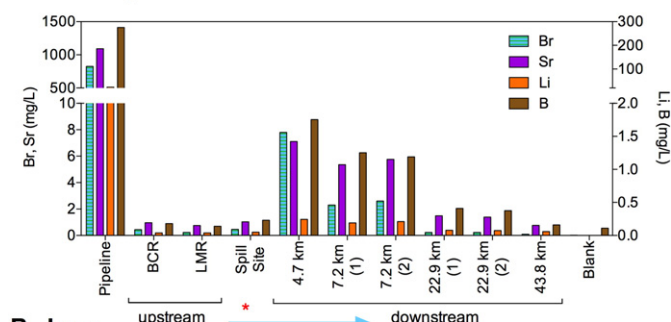


Fig. 3. Chloride (Cl), sodium (Na), and non-volatile dissolved organic carbon (NVDOC) concentrations (mg/L) in February 2015 (A) and June 2015 (B) in the brine pipeline sample and in water samples from surface water collected along Blacktail Creek and the Little Muddy River, as shown in Fig. 2. Field duplicate samples were collected at sites 7.2 km and 22.9 km downstream of the Spill Site and are indicated by (1) and (2). ND = not determined.

A. February



B. June

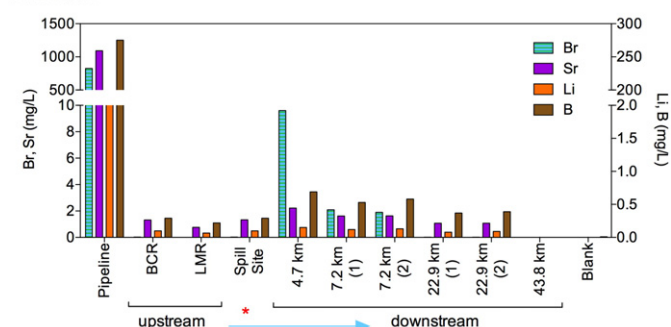


Fig. 4. Bromide (Br), strontium (Sr), lithium (Li), and boron (B) concentrations in February 2015 (A) and June 2015 (B) in the brine pipeline sample and in water samples from surface water collected at the sites shown in Fig. 2. Field duplicate samples were collected at sites 7.2 and 22.9 km downstream of the Spill Site and are indicated by (1) and (2). ND = not determined.

2016) as a result of a common source for the formation waters of ancient, evaporated seawater.

Other major anions in this system include SO_4 , which is naturally high in surface waters of the region (approximately 500 to 1500 mg SO_4/L in the upstream background samples) and HCO_3^- (410–635 mg HCO_3^-/L in the upstream samples) (Table S9). Neither of these species were present downstream from the spill at concentrations substantially different from background concentrations. Sulfate accounted for all of the dissolved sulfur detected in samples from all sites within analytical uncertainties. Nitrate and phosphate were below detection in all samples. Cations of calcium (Ca), magnesium (Mg), and potassium (K) were present at very high concentrations in the pipeline sample (Table S8) and were detectable at 1 to 3 times the background concentrations in Blacktail Creek at 4.7 km in February 2015 (Table S9). By June 2015, only K was still detectable above background concentrations at this location. The ammonium concentration measured in the pipeline sample was 2500 mg N/L (Table S8); unfortunately no surface water samples were able to be analyzed for NH_4 in February from Blacktail Creek.

Calcium and carbonate concentrations in water samples collected along Blacktail Creek were near saturation with respect to calcite in February (Table S11, Fig. S3). Stream-water samples were highly supersaturated with respect to calcite in June, consistent with findings from Lauer et al. (2016) for stream-water samples collected a few weeks later downstream from the Spill Site. Aqueous compositions along Blacktail Creek show partial pressures of CO_2 supersaturated with the atmosphere by a factor of 20 or more in February. In winter the ice-cover prevents atmospheric exchange, allowing accumulation of CO_2 which, in turn, contributes to conditions favorable to dissolution of carbonate minerals. The P_{CO_2} values in June were close to equilibrium with the atmosphere (saturation ratios of 0.6–2), indicating that rates of exchange with the atmosphere were commensurate with rates of CO_2 production and consumption in the stream. It is possible that calcite or aragonite (saturation index for aragonite is 0.14 units lower than that for calcite) precipitation in the summer could result in accumulation

of a reservoir of Sr in the sediments, the dissolution of which could provide a source to aquatic communities trapped under ice in the winter.

3.2. Minor and trace elements

The pipeline sample contained elevated concentrations of many other elements that are typically present at low concentrations in surface waters in the region; manganese and iron concentrations, for example, were 5.6 mg/L and 34 mg/L, respectively (Table S8). Manganese concentrations downstream from the Spill Site in February 2015 were approximately double the background upstream concentrations and reached a maximum value of 470 $\mu\text{g}/\text{L}$ (Table S9), which is substantially greater than the Maximum Contaminant Level (MCL) of 50 $\mu\text{g}/\text{L}$. Manganese and iron are expected to undergo sorption onto stream sediments and oxidation-reduction reactions throughout the water column. The temporal and spatial scales over which these reactions operate to retain or remobilize these elements in Blacktail Creek are unknown. Concentrations of many trace inorganic elements were quantified in stream-water samples (Table S9). The extent to which these elements are derived from the wastewater spill as compared to other anthropogenic (e.g., agriculture) and natural sources was not determined.

Strontium and barium concentrations along Blacktail Creek were elevated downstream from the Spill Site in both February and June (Fig. 4, Table S9). These elements have previously been reported to be useful tracers of UOG wastewater impacts from Marcellus Shale development (e.g. Akob et al., 2016; Brantley et al., 2014). Strontium concentrations 4.7 and 7.2 km downstream from the Spill Site were 7–9 times the BCR concentration in February and twice the BCR concentration in June. Barium concentrations 4.7 and 7.2 km along Blacktail Creek were two to three times the corresponding concentrations at the BCR site in both February and June. Aqueous chemical compositions of stream-water samples collected in February at all sites along Blacktail Creek indicated supersaturation with respect to barite (BaSO_4), with somewhat higher supersaturations downstream from the Spill Site (Table S11, Fig. S3). By June 2015, only water samples collected downstream from the Spill Site were supersaturated with respect to barite. Lauer et al. (2016) also previously reported that water in Blacktail Creek downstream from the Spill Site was supersaturated with respect to barite. Radium co-precipitates readily with barite (e.g., Brandt et al., 2015; Curti et al., 2010). Therefore, barite precipitation in the wastewater flowing through the pipeline and water downstream from the spill could result in a reservoir of wastewater-derived Ba and Ra in the sediments that could continue to supply these elements to benthic communities and stream water after the pulse of aqueous spill-derived Ba and Ra has been transported downstream.

3.3. Isotope signatures

The $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic composition of the pipeline wastewater was distinctly more radiogenic than the Sr isotopic composition in water samples collected from background sites BCR and LMR (Fig. 5). The degree to which the composition of the single sample of pipeline fluid we were able to obtain is representative of the composition of the millions of liters spilled is unknown, but likely there was some variability over time in the composition of fluid in the pipeline. To estimate the contribution of the pipeline fluid to the various samples, an $^{87}\text{Sr}/^{86}\text{Sr}$ mass balance mixing models between the pipeline fluid and the Blacktail reference site (BCR) was produced (detailed in SI Methods). Strontium compositions in water samples collected along Blacktail Creek 4.7 and 7.2 km downstream of the spill both in February and June 2015 plot closely along mixing lines between the pipeline spill sample and the corresponding BCR sample (Fig. 5), as did the July 2015 samples collected by Lauer et al. (2016) (although the composition of an upstream site was not provided in that study). Deviations between the observed compositions and the actual compositions likely result primarily from

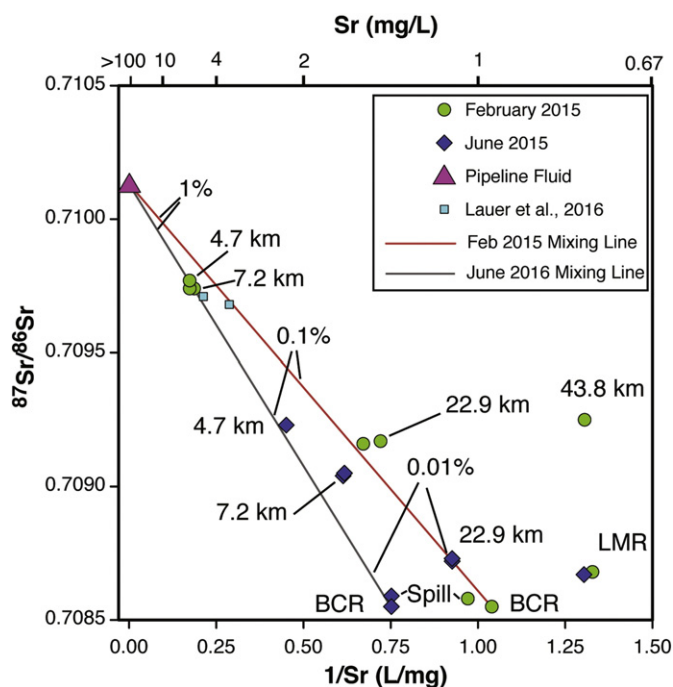


Fig. 5. Ratios of $^{87}\text{Sr}/^{86}\text{Sr}$ plotted against the reciprocal of the strontium concentration (mg Sr/L) for aqueous samples from February 2015 (green) and June 2015 (blue) collected at sites shown in Fig. 2. Also shown are data from the site from July 2015 by Lauer et al. (2016). Lines from mixing calculations are shown along Blacktail Creek (squares). External precision of the isotopic measurements ($2s = 0.000013$) is smaller than the symbol size.

variations over time in the background composition, as can be seen by comparing the locations of the BCR compositions in February and June 2015 (Fig. 5). Results from the mixing calculations (Table S10) indicate that 0.3 and 0.09% of the Sr in water collected from Blacktail Creek 4.7 km downstream of the Spill Site in February and June 2015, respectively, was derived from the spill. The relative contribution of pipeline fluid at the spill site was negligible, again suggesting that local remediation activities such as groundwater pumping limited movement of pipeline fluid into the stream at this location close to the point of the fluid release. These estimated contributions compare well with mass balance mixing models using Cl data (Table S10). Similarly, water collected from Blacktail Creek 7.2 km downstream from the Spill Site in February and June 2015 had 0.3% and 0.064%, respectively, of its Sr

derived from the spill. The Sr compositions of water samples collected from the 22.9 km downstream site, in February 2015, reflect mixing of water from the reference sites (LMR and BCR) and the spilled pipeline fluid. The isotopic composition of these samples was notably more radiogenic than the background samples from either source, consistent with a contribution from water from the spill.

3.4. Semi-volatile and extractable hydrocarbons in water and sediment

In February 2015, the field team reported visible oil sheen on the water under the ice, downstream from the pipeline break. The semi-volatile hydrocarbon analyses done on unfiltered samples showed the presence of low levels of alkylbenzenes and methyl-, dimethyl-, and trimethyl-naphthalenes in unfiltered samples downstream from the spill (Table 1). Concentrations were in the range 1 to 5 $\mu\text{g/L}$. 1-Methyl-naphthalene was detected the farthest downstream at 22.9 km. Extractable hydrocarbon analyses of filtered samples, however, showed no detectable concentrations of hydrocarbons (Fig. S4) and no measurable concentrations of UOG production additives. This method has been used previously to identify organic compounds in wastewater from UOG production by this USGS laboratory (Engle et al., 2016; Orem et al., 2014) and by others investigating produced water chemistry in shale-gas wells in Colorado, Pennsylvania, Texas and New Mexico (Lester et al., 2015; Maguire-Boyle and Barron, 2014). There was, however, an unresolved complex mixture (UCM) evident in the chromatograms from all sites along Blacktail Creek in February, that may indicate a mixture of hydrocarbons (straight and branched alkanes and alkenes and aromatics), but at low levels. The UCM was present both upstream and downstream from the spill and is thus not likely to be linked to the wastewater spill and may be due to background organic compounds present naturally in the watershed, or possibly hydrocarbons from general oil and gas production in the region.

These results indicate that the semi-volatile hydrocarbons that were measured at very low levels in the river downstream from the spill at the time of the sampling may have been associated with particulate matter in the creek since they were not detected in filtered samples. Sorption of hydrophobic organic compounds, including naphthalene onto soil and sediments, is well-documented (Eadie et al., 1996; Kile et al., 1995; Moyo et al., 2014) and known to be enhanced at lower temperatures (Piatt et al., 1996). The sediment extracts of soils done in February 2015 contained traces of naphthalene, methyl-naphthalenes, and other polycyclic aromatic hydrocarbons but they were below the reporting limits in all samples. Sorption processes might also explain the lack of detection of UOG additives in the river downstream from the spill (the wastewater flowed overland or through sediments for

Table 1

Semi-volatile organics observed in water samples from Blacktail Creek and Little Muddy River. Compounds which were tentatively identified via automated search against NIST libraries, but were not quantified are indicated by "+"; compounds not detected are indicated by "-". Quantified compounds are in $\mu\text{g/L}$ of the compound and were identified against deuterated internal standards. Field duplicate samples were collected at sites 7.2 and 22.9 km downstream of the Spill Site and are indicated by (1) and (2).

	BCR	LMR	Spill Site	4.7 km	7.2 km (1)	7.2 km (2)	22.9 km (1)	22.9 km (2)	43.8 km	Blank
1,3,5-Trimethylbenzene	—	—	—	0.390	1.490	—	—	—	—	—
1,2,3,5-Tetramethylbenzene	—	—	—	+	+	+	—	—	—	—
1,2,3,4-Tetramethylbenzene	—	—	—	3.64	4.76	4.56	—	—	—	—
n-Ethyl-n,n-dimethylbenzene	—	—	—	—	+	+	—	—	—	—
Naphthalene	—	—	—	—	—	—	—	—	—	—
2-Methylnaphthalene	—	—	—	—	—	—	—	—	—	—
1-Methylnaphthalene	—	—	—	1.71	2.17	2.12	0.13	0.10	—	—
Ethyl-naphthalene	—	—	—	—	—	—	—	—	—	—
2,6;2,7-Dimethylnaphthalene	—	—	—	+	+	+	+	+	—	—
1,3;1,7-Dimethylnaphthalene	—	—	+	+	+	+	—	—	—	—
1,6-Dimethylnaphthalene	—	—	—	+	+	+	—	—	—	—
2,3; 1,4; 1,5-Dimethylnaphthalene	—	—	+	—	—	—	+	+	—	—
1,2-Dimethylnaphthalene	—	—	—	—	—	—	—	—	—	—
1,8-Dimethylnaphthalene	—	—	—	—	—	—	—	—	—	—
1,4,6 Trimethylnaphthalene	—	—	—	+	+	+	+	+	—	—
2,3,6 Trimethylnaphthalene	—	—	+	+	+	+	+	+	—	—
1,6,7 Trimethylnaphthalene	—	—	+	+	+	+	+	+	—	—

10s of meters before entering the stream), although the lack of any pipeline sample for organics analyses limits what can be said about the potential fate of organic compounds in the wastewater.

3.5. Light (C_1 – C_6) hydrocarbons in surface water

Light hydrocarbons were at low concentrations in Blacktail and Little Muddy Creek (Fig. 6, Table S12), but show a distinct thermogenic hydrocarbon signature. Total concentrations in both upstream and downstream locations were higher in February than in June, probably due to the ice cap over the rivers that would have restricted atmospheric venting. Methane is the most prevalent compound in all light hydrocarbon samples, and total C_1 – C_6 concentrations vary between 39.8 and 91.3 nmol/kg in February and 0.91 and 37.7 in June. In February 2015, the 7.2 km site had the highest ethane and propane concentrations (1 to 2 orders of magnitude higher than BCR). The ratios of the constituents of the dissolved hydrocarbons measurements can be used to infer the origins of the gases in the sample. Biogenic gas is typically dominated by methane, with trace amounts of heavier alkanes (~ 1 mol% or less); in contrast, thermogenic gases can have significant amounts of higher alkanes in stepwise decreasing abundances in a cracking pattern produced by thermal maturation (Schoell, 1983; Taylor, 2000). The ethane/methane molar ratio increases to 5.74×10^{-1} at 7.2 km from 3.12×10^{-3} at BCR, with concomitant increases in the ratios of methane to higher alkanes, giving a distinct thermogenic signature to the light hydrocarbon ratios that extends to *n*-hexane, the heaviest alkane reported. The ratios of ethane/methane, propane/ethane, and *n*-butane/propane were maintained in downstream samples after the confluence of the Blacktail Creek and Little Muddy River. Compared to the BCR and LMR, the concentrations of hydrocarbons, particularly ethane and unsaturated hydrocarbons were generally elevated downstream, including the 43.8 km Site. The ratios

of propane/ethane and *n*-pentane/*n*-butane were also similar at the 22.9 km site. Compared to the BCR and LMR, the concentrations of hydrocarbons, particularly ethane and unsaturated hydrocarbons were generally elevated downstream, including the 43.8 km site. The ratios of propane/ethane and *n*-pentane/*n*-butane were also similar at the 22.9 km site.

In June 2015, the Spill Site, 4.7 km, and 7.2 km had the highest methane and ethane concentrations. Although 7.2 km had the highest ethane and propane concentrations in water from the sites sampled in June, the ratios of ethane, propane, and other higher hydrocarbons to methane at the 7.2 km site were lower than in February due to the relatively large amount of methane present (ethane/methane: 1.11×10^{-2} , propane/methane: 5.28×10^{-3}). The ratios of propane/ethane and *n*-butane/propane were similar to those observed at the site in February and distinct from those observed in the background sites, suggesting that there was a common, lingering source of these compounds at 7.2 km between the two sampling dates. In February, the downstream hydrocarbon concentrations and ratios show dilution and transport of a common source (or set of sources) of thermogenic hydrocarbons. The persistence of these was likely enhanced by the limited ventilation through the iced covered river, higher solubility at low temperatures, and limited microbiological activity in winter. In contrast, the June downstream concentrations of C_2 – C_6 hydrocarbons were low enough that exogenous sources (from microbial activity and water in contact with ambient air with mixing ratios of less than a ppbv) obscure the ratios of compounds that might be transported downstream and diluted from the 7.2 km site during the June sampling event. The ratios and concentrations upstream at 4.2 km and the Spill Site were discordant with those observed at 7.2 km (and downstream in February). If the light hydrocarbons were ultimately sourced from the pipeline spill, this could indicate that the upstream light hydrocarbons were modified or attenuated by the remediation activities, and that they were sorbed to in the riverbed or shallow groundwater around the pipe and are slowly leaving via the discharge into the river.

These light hydrocarbon measurements are interesting because they show that releases may be traced considerable distance downstream and may linger in the environment for considerable time even though they are highly volatile. Although the controls on the concentrations and ratios of light hydrocarbons at low concentrations in water are not well understood, the differences between upstream, spill, and far downstream sites show that this technique has promise for identifying the extent and compositional origin of UOG materials released into the environment. The concentrations of alkenes may also be indicators of microbial activity, for example the high levels of ethene, a compound released by algae as a byproduct of sulfur reduction (Plettner et al., 2005).

3.6. Sediment chemistry

Labile Ba and Sr concentrations extracted from sediments collected along Blacktail Creek in June 2015 were higher downstream from the Spill Site than upstream (Fig. 7a). Labile sediment-bound Ba and Sr were assayed using short-term, dilute hydrochloric acid extractions (methods detailed in the SI). Sediments collected directly from the Spill Site had labile Ba and Sr concentrations similar to sediments collected upstream. This is likely a result of the remediation operations that had been conducted at the Spill Site, similar to the results seen in the water samples. The isotopic composition of labile Sr extracted from sediments collected downstream in June 2015 exhibited a significant contribution of Sr from the pipeline fluid (Fig. 7b), especially at the 4.7 km site. This observation supports the hypothesis that the small increase in labile Sr on sediments downstream from the spill results from retention of Sr and, likely, Ba from the spill on the sediments.

Radium activities in sediments from Blacktail Creek and the Little Muddy River ranged from a background of about 10–20 Bq/kg in sediments collected upstream at BCR to 464 Bq/kg in sediments collected from the Spill Site in June 2015 (Fig. 8, Table 2). Ratios of $^{226}\text{Ra}/^{238}\text{U}$

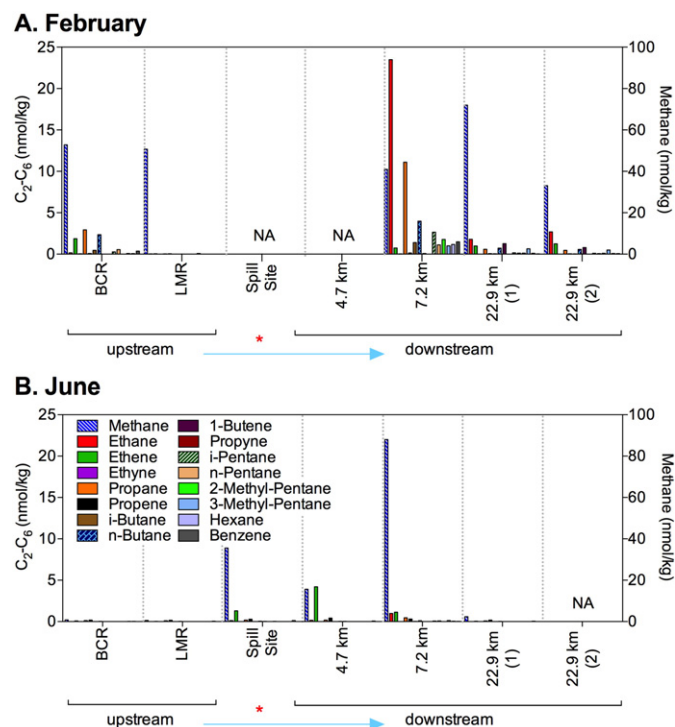


Fig. 6. Low level hydrocarbon concentrations in February 2015 (A) and June 2015 (B) in water samples from surface water collected along Blacktail Creek and the Little Muddy River, as shown in Fig. 2. No samples were analyzed from the Spill Site and 4.7 km collected in February due to observed liquid hydrocarbon sheen on the water surface that precluded trace hydrocarbon analysis. Concentrations are expressed in nanomoles of the specific compound per kilogram water. Field duplicate samples were collected at the 22.9 km site, indicated by (1) and (2). NA = not analyzed.

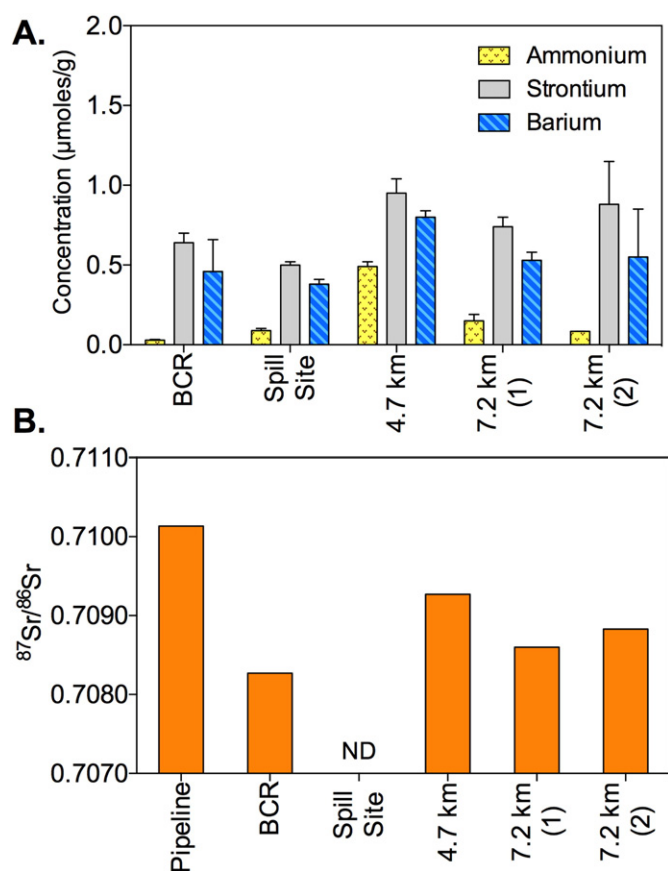


Fig. 7. (A) Potassium-chloride-extractable ammonium and hydrochloric-acid-extractable strontium and barium concentrations from sediments collected along Blacktail Creek in June 2015. (B) Strontium isotopic ratios in HCl extracts of the sediments collected along Blacktail Creek and the pipeline brine aqueous sample. Field duplicate samples were collected at site 7.2 km and are indicated by (1) and (2). External precision of the isotopic measurements ($2s = 0.000013$) is too small to be plotted. ND = not determined.

equal to 1.0 (within measurement error) were observed in sediment samples collected upstream from the Spill Site suggesting secular equilibrium in these samples. Given that secular equilibrium in the ^{226}Ra – ^{238}U system requires several thousand years, it is likely that the Ra content of these samples represents naturally occurring background concentrations of Ra. Sediments collected in February 2015 had slightly elevated $^{226}\text{Ra}/^{238}\text{U}$ ratios over secular equilibrium at the Spill Site. However, it is important to note the February Spill Site sample may not be representative of actual spill concentrations at this location because of remediation efforts, including soil removal, which had already

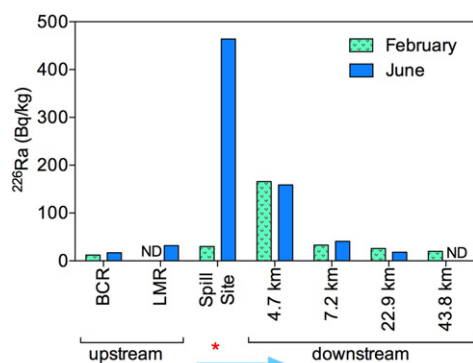


Fig. 8. Radium-226 activities in river sediment in February 2015 and June 2015 collected along Blacktail Creek and the Little Muddy River, as shown in Fig. 2. “ND” indicates no data were collected.

occurred at the spill location. Additionally, comparison of low activity samples such as these is difficult as the differences of grain size and geometry, interference peaks of other radioactive material, and low counts create greater uncertainty compared to higher activity samples. The $^{226}\text{Ra}/^{238}\text{U}$ ratios downstream at 4.7 km in February 2015, had a 9-fold excess of ^{226}Ra over secular equilibrium and suggests that the spill had resulted in contamination of these sediments with ^{226}Ra . These observations are consistent with those of Akob et al. (2016) who found that $^{226}\text{Ra}/^{238}\text{U}$ activity ratios in sediments at a wastewater injection facility could be used to detect wastewater spills. Samples collected in February 2015 farther downstream showed no significant elevation of ^{226}Ra over that in secular equilibrium with ^{238}U .

Sediments collected at the Spill Site in June 2015 exhibited higher concentrations of Ra in excess of secular equilibrium, compared with February 2015 samples (Table 2), suggesting additional input of contaminated water or sediments at the site. Furthermore, sediments collected at 4.7 km downstream and at 7.2 km downstream from the Spill Site had a 14-fold and 5.5-fold excess of ^{226}Ra over secular equilibrium, respectively. Lauer et al. (2016) found that sediments collected downstream from the Spill Site in July 2015 had total Ra [^{226}Ra plus ^{228}Ra] activities in the range 550 to 4700 Bq/kg whereas water samples had total Ra activities of 0.3 Bq/L. Sediments from Blacktail Creek with excess ^{226}Ra also had $^{228}\text{Ra}/^{226}\text{Ra}$ ratios (Table 2) lower than the value of 1.1 determined on sediments from Blacktail Creek upstream from the Spill Site by Lauer et al. (2016), consistent with contributions from Bakken brines, which have $^{228}\text{Ra}/^{226}\text{Ra}$ ratios in the range 0.4–0.5 (Lauer et al., 2016). However, it should be noted that ^{228}Ra has a 5.75 year half-life and our sediment samples were measured up to a little over a year after they were collected. Ratios in our sediment samples may be lower than those determined by Lauer et al. (2016) because our activity ratios have not been corrected for ^{228}Ra decay between when our sediment samples were collected and when they were analyzed.

The chemical form of excess Ra in wastewater-contaminated sediments is unknown. As discussed previously, Ra incorporated into barite is one potential form of sediment-bound Ra. In addition, Ra sorption can occur on sediments exposed to elevated dissolved concentrations of Ra. However, it has been noted by others (Harto et al., 2014; Kraemer and Reid, 1984; Landa and Reid, 1983; Lauer et al., 2016; Nelson et al., 2015) that the degree of Ra sorption to sediment and soil depends on both the quantity and quality of the water present in the sample (such as salinity and chemistry) and the type and character of the sediment or soil (e.g., grain size, organic matter content, cation exchange capacity). The salinity of the brines can inhibit Ra sorption to the soil (which would have a higher potential of occurring close to the spill source where the salinity is greater). Downstream from the spill, the brine becomes diluted with freshwater from surface and groundwater, which could favor Ra sorption onto the sediments. Determining the chemical form of Ra in the sediments is an important topic of further research.

The increases in ^{226}Ra activities in sediment samples collected along Blacktail Creek between February and June 2015, could be a reflection of transport of contaminated material downstream, changing site conditions, and remediation efforts at the spill site. Downstream transport of particle-associated constituents are hypothesized to occur through two potential mechanisms: 1) fluvial transport and storage of fine sediment with precipitates and sorbed constituents, and 2) transport of contaminated groundwater through the channel bed sediments and the hyporheic zone. Field conditions in February were cold and the stream was frozen (Fig. S1), possibly limiting input of contaminated soil to the stream. Remediation efforts were also fully underway with significant soil around the spill having been removed, limiting the availability of contaminated material for transport. In June 2015, sampling was conducted after the spring snowmelt which could have resulted in increased groundwater transport, thereby contaminating soils, and enhancing Ra sorption onto fluvial sediment. These results are consistent with work done on Ra activities conducted along Blacktail Creek by Lauer et al. (2016).

Table 2
Uranium and radium activities^a in sediments from background, Spill Site, and downstream locations.

Location	Date	²³⁸ U (Bq/kg)	²²⁶ Ra (Bq/kg)	²²⁸ Ra (Bq/kg)	²²⁸ Ra/ ²³⁸ U	²²⁸ Ra/ ²²⁶ Ra
BCR	2/11/15	20 ± 7	12 ± 4	ND ^b	0.6 ± 0.3	–
LMR	No sample					
Spill Site ^d	2/11/15	12 ± 7	30 ± 4	ND ^b	2.6 ± 1.5	–
4.7 km	2/11/15	18 ± 6	166 ± 3	149 ± 7	9.2 ± 2.9	0.90 ± 0.05
7.2 km	2/10/15	29 ± 5	33 ± 4	ND	1.2 ± 0.3	–
22.9 km	2/10/15	30 ± 7	26 ± 5	ND	0.8 ± 0.2	–
43.8 km	2/10/15	BD ^c	20 ± 1	BD	–	–
BCR	6/17/15	9 ± 4	17 ± 2	ND	1.8 ± 0.8	–
LMR	6/17/15	29 ± 3	32 ± 1	21 ± 1	1.1 ± 0.1	0.66 ± 0.11
Spill Site	6/17/15	26 ± 9	464 ± 7	290 ± 1	18 ± 4	0.62 ± 0.01
4.7 km	6/17/15	12 ± 6	159 ± 2	133 ± 2	14 ± 1	0.83 ± 0.02
7.2 km	6/17/15	8 ± 2	41 ± 2	33 ± 1	5.5 ± 1.7	0.74 ± 0.09
22.9 km	6/18/15	11 ± 8	18 ± 5	ND	1.6 ± 1.2	–
43.8 km	No sample					

^a Activity in becquerels (disintegrations per second) per kilogram sediment dry weight. Concentrations can be calculated from the formula: $C_i = (A_i t_{1/2,i}) / ((N_A)(\ln 2))$, where i refers to the specific radionuclide, C_i the concentration (moles/kg sediment), A_i the activity, $t_{1/2,i}$ the half-life (seconds), N_A Avogadro's number (dimensionless), and $\ln 2$ is the natural logarithm of 2.

^b ND = not determined.

^c BD = below detection, could not be quantified because of low activity.

^d Coarse gravelly sample; may effect concentrations.

Labile sediment bound ammonium concentrations were assayed using short-term potassium chloride extractions (as described in the SI Methods) on sediment samples collected in June 2015. Sediment-bound ammonium concentrations 4.7 km downstream from the Spill Site were over 10 times those observed at the background site (BCR) (Fig. 7A, Table S13). Sediment-bound ammonium concentrations were somewhat elevated compared to the background site at the Spill Site and 7.2 km downstream from the Spill Site. This pattern is similar to the pattern in sediment-bound Ba and Sr, consistent with the pipeline spill being the source of all three constituents (Fig. 7A). However, contributions to the observed pattern from variability in natural and anthropogenic sources along Blacktail Creek cannot be ruled out without additional research.

3.7. Potential endocrine disruption activity

No estrogenic receptor activity was noted for the mammalian assays in water downstream from the Spill Site, compared with water collected upstream, in either February or June 2015 (Fig. 9a). However, estrogen receptor inhibition was substantially greater at 7.2 km downstream in February 2015 compared to the upstream waters at BCR. These differences were still measurable but less pronounced in June 2015 (Fig. 9b). However, the water sample from Little Muddy River also showed some estrogen receptor inhibition in the same range as the June 2015 samples, indicating the June observations may be within natural variations in these waters.

Estrogenicity using yeast reporter strains was noted in samples collected at a number of sites during February and June (Fig. 9c). Estrogenicity was detected in samples from all sites evaluated during the February collection. The process control was always below detection. Net estrogenicity was greatest at the site 4.7 km downstream from the spill (2.13 ± 0.11 ng/L). Modest estrogenic activity was observed both up- and downstream from the Spill Site. Calculated estrogen equivalents (EEQ) ranged from 1.30–2.13 ng/L across the downstream site gradient from BCR to 4.7 km. In general, estrogenicity was higher in February compared to June. The maximum observed EEQ in June was 0.54 ± 0.11 ng/L at BCR, upstream from the spill location on Blacktail Creek. Endocrine disrupting activity has been associated with UOG spills in Colorado and West Virginia (Kassotis et al., 2014; Kassotis et al., 2016b); to our knowledge, this is the first report of EDC activity associated with UOG spills in ND. Although these results indicate that a full understanding of the endocrine disrupting chemical (EDC) activity

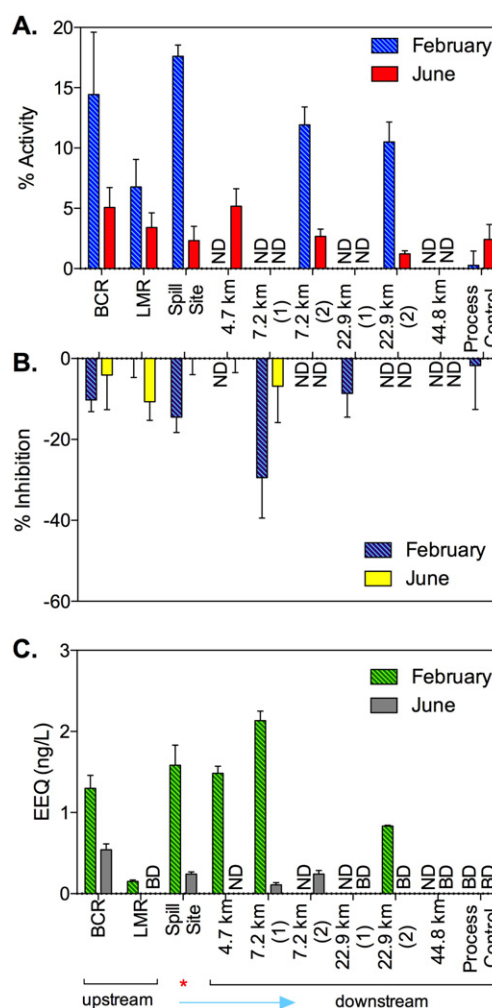


Fig. 9. Estrogen receptor (A) activity and (B) inhibition of surface water samples collected at sites indicated via mammalian reporter gene assay. (C) Estrogen equivalents (EEQ, relative to 17 β -estradiol) of OASIS HLB extracted water samples using the bioluminescent yeast estrogen screen (BLYES). Values are mean of triplicate reads; error bars indicate standard error of the mean. BD = below detection. ND = not determined (sample not analyzed). Field duplicate samples were collected at sites 7.2 and 22.9 km downstream of the Spill Site and are indicated by (1) and (2). The duplicate sample from 4.7 km was not evaluated due to sample loss during shipping.

of water downstream from these types of spills requires further study, the elevated EDC activity, specifically inhibition of estrogen receptor activity, downstream from the spill site in February is consistent with previous reports of an association between EDC activity in water and UOG activities (Kassotis et al., 2014; Kassotis et al., 2016b). Previous work by others has shown antiestrogens can negatively impact aquatic organisms, and in the current study we found moderate antiestrogenic activity within a potential bioactive range (Madureira et al., 2015; Roepke et al., 2005).

3.8. Aquatic health studies

Ninety-six hour survival of early life stage Fathead Minnows (FHM) downstream from the wastewater pipeline rupture was significantly reduced at 1 of 4 experimental sites approximately 6-months after the spill was detected. Survival of <48 h aged FHM after 96 h was 89.2% at BCR, 94.7% at LMR, 89.7% at the Spill Site, 89.2% at 4.7 km, 2.5% at 7.2 km, and 74.2% at 22.9 km (Table S14). The reduction in survival observed at 7.2 km is statistically significant at $p \leq 0.05$. Mortality of two resident Madtom Catfish (*Noturus* sp.) was observed at 7.2 km at 72 h and an oily rainbow-colored sheen was observed on the water surface at 96 h (Fig. S5), although the relationship between this observation and fish survival is unknown. Although no systematic native fish sampling was conducted, live resident fish were observed at all sites, with the exception of the 7.2 km site where no live resident fish were observed (Fig. S5b and c). There was no statistical difference between survival at the LMR and the 22.9 km downstream site.

Large diel fluctuations in dissolved oxygen and temperature were recorded at all sites in June 2105; oxygen saturation ranged from <10% to over 200% (Fig. S6), and water temperatures ranged from 15 to 28 °C, except at site 7.2 km. At the 7.2 km site declining temperatures were observed at 72 and 96 h, dropping to 7.95 °C. Total dissolved salt (TDS) concentrations, hardness, and SO_4 concentrations were generally consistent among sites. Concentrations of Cl and bicarbonate were stable at all sites except at 7.2 km, where they increased from 194 to 568 mg/L and from 41.6 to 727 mg CaCO_3 /L, respectively. To a lesser degree, Cl but not bicarbonate increased at the 4.7 km site, where 316 and 499 mg/L were measured at 0 and 96 h respectively with a slight decrease to 308 mg/L at 24 h (Table S15).

Reduced temperatures and increased Cl and HCO_3 concentrations suggests a pulsed upwelling of groundwater into the stream at the time that mortalities were observed. Variations in stream discharge observed during our field investigations and are consistent with the observations of paleochannels by environmental remediation consultants working at the site (H. Rhodes, personal communication, June 2015); these paleochannels are highly conductive and could act as conduits of spill contaminated groundwater into the surface water. The changes observed in water chemistry were accompanied by a decrease in surface water flow, and highlight the need for further study to assess the hydrologic conditions and potential contaminant pathways at the site.

Changes in water chemistry and visual observations concurrent with observed mortalities were increased Cl and bicarbonate concentrations, decreased water temperature, and a rainbow-colored oily sheen on the water's surface. The median lethal concentration (LC 50) of Cl and HCO_3 to FHM is approximately 4000 mg Cl/L and 1250 mg HCO_3 /L respectively in laboratory studies (Harper et al., 2014; Mount et al., 1997). But in waters with high hardness, calcium may have an ameliorating effect on ion toxicity (Soucek et al., 2011). Under the hardness conditions found in Blacktail Creek, the increased concentrations of Cl and HCO_3 alone did not appear sufficiently high to induce acute toxicity at the 7.2 km site.

The 2500 mg N/L NH_4 concentration (as defined above as total NH_4) in the pipeline spill fluid had the potential for causing chronic or even acute toxicity to fish and aquatic life. The toxic form of NH_4 is the aqueous NH_3 species, sometimes referred to as “un-ionized ammonia”. The U.S. EPA acute NH_4 criterion for the protection of aquatic life is 17 mg N/L and the chronic NH_4 criterion is 1.9 mg N/L (U.S. EPA,

2013). Note that the U.S. EPA (2013) term “ammonia” refers to what we call “total dissolved ammonium” (NH_4). Ammonium concentrations of 10 mg N/L were measured at the 7.2 km site in February (Table S9) but aqueous NH_3 concentrations were <0.1 mg N/L owing to the low pH values (Table S11). Lauer et al. (2016) reported NH_4 concentrations in water samples collected in July 2015 from Blacktail Creek near the Spill Site of 17–21 mg N/L, equal to or exceeding the U.S. EPA criterion for acute toxicity. Aqueous NH_3 concentrations could not be computed because Lauer et al. (2016) did not report pH values or temperatures for individual samples. After completion of the experiment with FHM described above, NH_4 concentrations were determined in water samples collected at BCR, LMR, the Spill Site, and at 4.7 km, 7.2 km, and 22.9 km downstream. Concentrations at all sites were 0.1 mg N/L or less except the 7.2 km site, which had an NH_4 concentration of 3.4 mg N/L (Table S16). This concentration exceeds the U.S. EPA criterion for chronic toxicity to aquatic life. Thurston et al. (1983) reported acute aqueous NH_3 toxicity to FHM ranging from 0.75 to 3.4 mg N/L; the toxicity was reduced as temperature increased to 23 °C, the upper temperature limit of the study. At the pH and temperature at site 7.2 km (8.83, 27 °C), the NH_4 concentration of 3.4 mg N/L corresponds to a concentration of aqueous NH_3 of 0.85 mg N/L, within the range of concentrations Thurston et al. (1983) found to cause acute toxicity to FHM. The U.S. EPA criterion for acute toxicity at a temperature of 27 °C and pH of 8.8 is 0.44 mg N/L of total NH_4 (U.S. EPA, 2013). Thus, these measurements, under the conditions observed at our site, show that chronic or even acute toxic levels of NH_4 could have been reached in Blacktail Creek.

In situ experiments take advantage of diel fluctuations in physical and chemical conditions that cannot be recreated in the laboratory but are typical in prairie stream conditions (Farag et al., 2014). As a result, these experiments provide relevant information about the resiliency of fish in Blacktail Creek and the Little Muddy River six months after the UOG wastewater pipeline rupture, and during continued remediation efforts. Early life stage FHM did not survive 96 h at the 7.2 km site. Almost all of the mortalities from this site were observed 24 h following the discovery of dead resident fish at the site. Diel fluctuations in DO were large at all sites measured and neither the BCR nor the 7.2 km sites went anoxic (Fig. S6), yet significant mortality of FHM was observed only at the 7.2 km site, and mortalities of resident madtoms occurred at the same site within 24 h of the experiments with the FHM. These observations suggest that changes in DO were not the cause of death in the madtoms or FHM.

Laboratory invertebrate health studies showed an inconclusive impact on amphipods. Differences in survival, growth (average mg per individual), and total biomass (total mg per replicate) of amphipods, midges, and mussels among sites were evaluated by one-way analysis of variance of rank-transformed data, with differences among means evaluated using Tukey's test. Survival of all three species was unaffected. Growth of midges and mussels and biomass of all three species differed significantly among sites, but sites downstream from the spill location generally did not show significant reductions in these endpoints relative to reference sites (Table S17). Overall, there was a non-significant trend of lower growth and biomass of amphipods at two sites downstream from the Spill Site (e.g., growth amphipod BCR 0.76 mg $\pm$ 0.02 vs. Spill Site 0.67 mg $\pm$ 0.04 and 4.7 km 0.59 mg $\pm$ 0.07) and to a lesser extent the 7.2 km site (0.73 mg $\pm$ 0.07). Although these results do not demonstrate strong or consistent toxic effects of sediments from reaches of Blacktail Creek or Little Muddy Creek affected by the spill, they do indicate that longer-term investigations of sediment toxicity after spill or release events are warranted.

4. Implications

Although there are critical knowledge gaps regarding the effects of contaminants released to the environment during UOG waste management activities, it is apparent from this study that the type of spill

(pipeline rupture), the constituents from the spill (brine with some hydrocarbons mixture), physiography, and location of the spill (discharge adjacent to a stream) will influence the environmental pathways and effects. This research advances our understanding and quantifies potential impacts through the analyses of Ra and Sr concentrations and isotopic compositions, trace inorganic and organic compounds, as well as endocrine disrupting effects and bioassays with model organisms. This set of analytical tools provides insights into potentials for human exposures. Concentrations of many wastewater-derived contaminants in stream water were several times background concentrations, but still relatively low compared to U.S. EPA drinking water standards. Nevertheless, wastewater-derived elements and radioisotopes partitioned onto sediments, potentially providing a long-term source of Ba, Ra, and other contaminants to aquatic life. Results from this study and Lauer et al. (2016) show that radium activities were significantly above the U.S. EPA action level for ^{226}Ra in surface soils, which should not exceed 5 pCi/g (185 Bq/kg). Episodic increases in NH_4 were at levels high enough to be toxic to aquatic life, particularly in the spring and summer when pH values increase during peak photosynthetic activity, shifting speciation in favor of the more toxic aqueous NH_3 form.

Potential health effects are indicated by fish bioassays, in which fish experienced mortality, and endocrine disrupting activity was observed downstream from the spill. There was a clear increase in antagonism below the spill site. This increased antagonism previously has been associated with UOG impacted waters (Kassotis et al., 2016a; Kassotis et al., 2014). The total estrogenicity measured above and below the spill site was modest compared to other locations in the U.S. where strain BLYES has been utilized to measure estrogen agonism. Measures of estrogenicity > 1 ng/L are typically more characteristic of waters impacted by wastewater reclamations discharge or animal feeding operations (Ciparis et al., 2012; Iwanowicz et al., 2016). Published accounts of a reasonable aquatic organism adverse effects threshold for estrogenicity range from 0.73 ng/L to 2 ng/L (Caldwell et al., 2012; Wu et al., 2014; Young et al., 2002). Conley et al. (2016) have suggested 1 ng/L as an adverse effects trigger value. A number of the February measures proximate to the Spill Site exceed this value suggesting the potential for adverse effects in resident aquatic organisms. The identity of these endocrine-active chemicals is not known based on the current analyses, but the bioassay endpoints clearly indicate their presence at biologically meaningful concentrations.

The results of this work suggest that whereas wastewater spills introduce both organic and inorganic constituents into the environment, elements from brines (such as NH_4 , Ra, Ba, and Sr) occur at higher concentrations and may persist longer in the environment due to partitioning onto sediment. This is an especially significant finding considering that 3 times as many brine spills were reported (2007–2015) in North Dakota as compared to oil spills during the same period.

Our observations show that initial remediation of the spill effectively removed some types of contamination while allowing others to persist. Temporal sampling indicates that contaminated groundwater and soil can reintroduce different contaminants at variable rates leading to potentially different site management concerns between initial contamination and long-term impact. Partitioning of chemicals onto the sediment limits movement of wastewater components downstream but could provide a long-term source of contaminants to aquatic organisms. Sediment-bound forms of elements like Ba, Sr, and Ra could include ion exchangeable species as well as elements incorporated into solid phases like calcite or aragonite, and barite. Carbonate minerals that form during the summer may dissolve in response to rising P_{CO_2} values during periods when ice-cover prevents atmospheric exchange, releasing co-precipitated contaminants. Increasing Ca concentrations in response to dissolution of carbonate minerals could perturb ion exchange equilibria, potentially mobilizing elements like Ba, Sr, and Ra. Understanding chemical forms of wastewater-spill derived contaminants retained in sediments is an important area of future research.

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.11.157>.

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Analysis of BTEX groundwater concentrations from surface spills associated with hydraulic fracturing operations

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Concerns have arisen among the public regarding the potential for drinking-water contamination from the migration of methane gas and hazardous chemicals associated with hydraulic fracturing and horizontal drilling. However, little attention has been paid to the potential for groundwater contamination resulting from surface spills from storage and production facilities at active well sites. We performed a search for publically available data regarding groundwater contamination from spills at U.S. drilling sites. The Colorado Oil and Gas Conservation Commission (COGCC) database was selected for further analysis because it was the most detailed. The majority of spills were in Weld County, Colorado, which has the highest density of wells that used hydraulic fracturing for completion, many producing both methane gas and crude oil. We analyzed publically available data reported by operators to the COGCC regarding surface spills that impacted groundwater. From July 2010 to July 2011, we noted 77 reported surface spills impacting the groundwater in Weld County, which resulted in surface spills associated with less than 0.5% of the active wells. The reported data included groundwater samples that were analyzed for benzene, toluene, ethylbenzene, and xylene (BTEX) components of crude oil. For groundwater samples taken both within the spill excavation area and on the first reported date of sampling, the BTEX measurements exceeded National Drinking Water maximum contaminant levels (MCLs) in 90, 30, 12, and 8% of the samples, respectively. However, actions taken to remediate the spills were effective at reducing BTEX levels, with at least 84% of the spills reportedly achieving remediation as of May 2012. Our analysis demonstrates that surface spills are an important route of potential groundwater contamination from hydraulic fracturing activities and should be a focus of programs to protect groundwater.

Implications: While benzene can occur naturally in groundwater sources, spills and migration of chemicals used for hydraulic fracturing activities have recently been thought to be a main source of benzene contamination in groundwater. However, there is little scientific literature to support that claim. Therefore, we accessed a publically available database and tracked the number of reported surface spills with potential groundwater impact over a 1-year period. Although the number of surface spills was minimal, our analysis provides scientific evidence that benzene can contaminate groundwater sources following surface spills at active well sites.

Supplemental Materials: Supplemental materials are available for this paper. Go to the publisher's online edition of the *Journal of the Air & Waste Management Association* for an illustration of the average concentration of each BTEX chemical from pooled sample measurements, and various metrics from all 77 spills analyzed in this study.

Introduction

Increases in the global demand for energy are driving advances in natural gas extraction techniques such as hydraulic fracturing and horizontal drilling (Kennedy, 2007). These two technologies make it economically feasible to recover unconventional oil and gas resources from coal beds, shale formations, and tight sand reservoirs. Although hydraulic fracturing has received recent attention, the technology has been in commercial use in the United States for exploration and extraction of crude oil since the 1940s (STRONGER, 2011). Hydraulic fracturing is a technology that relies on the high-pressure injection of water mixed

with a combination of chemicals and sand formulated to physically fracture subsurface reservoirs for the purpose of extracting oil and gas. Depending upon the type of geological formation and the depth associated with horizontal drilling, fracturing activities can take place anywhere from several hundred feet to several miles below the surface (ALL Consulting, 2009).

Public concerns have been expressed about drinking-water contamination from migration of chemicals used during the hydraulic fracturing process, as well as from the escape of methane from fractured rock and well casings (Dammel et al., 2011; Groat and Grimshaw, 2012; Osborn et al., 2011; Rozell and Reaven, 2012; U.S. EPA, 2011). However, strong scientific evidence to

support these concerns is lacking. To our knowledge, only one study has been published in the scientific literature evaluating the potential for groundwater contamination with methane (Osborn et al., 2011). These authors reported methane contamination of aquifers overlying the Marcellus Shale formation and noted that the contamination accompanied gas-well drilling and hydraulic fracturing activities in the area. However, the authors concluded that more research was still needed to clearly understand the mechanism of contamination (Osborn et al., 2011).

In November 2011, the U.S. EPA introduced a plan to examine methane contamination of drinking water in several drilling areas across the United States, including the Marcellus Shale; the results of this study are forthcoming (U.S. EPA, 2011). Regarding drinking water contamination from hydraulic fracturing fluids, a recent U.S. EPA study reported that two deep monitoring wells near an aquifer in Pavillion, Wyoming, tested positive for glycols, alcohols, and high levels of methane, all of which were thought to originate from hydraulic fracturing activity conducted below the aquifer. This was the first report of drinking-water contamination resulting from the migration of chemicals from a fractured formation, although, to date, confirmation of chemical migration remains in question and conclusions from this study are currently undergoing further evaluation (DiGiulio et al., 2011; McLernon, 2012).

Groundwater contamination may occur from various activities that take place at the ground surface before, during, and after a well is brought into production. In a recent article published in a nationally recognized water quality journal, it was noted that most water quality issues in the United States associated with hydraulic fracturing activities are the result of surface spills or leakage into the shallow water formations (Metzger, 2011). Only recently has the U.S. EPA announced its first proposal of a Quality Assurance Project Plan to analyze data from surface spills in states with both oil and gas production, such as Texas, Colorado, and Pennsylvania (U.S. EPA, 2012).

Based on our review, no study has been published in the peer-reviewed scientific literature that addresses the potential for groundwater contamination from surface spills associated with hydraulic fracturing activities (Groat and Grimshaw, 2012). Wells producing crude oil in addition to methane gas are a potential source of petroleum hydrocarbon release into groundwater via surface spills. Of particular interest is the release of benzene, toluene, ethylbenzene, and xylene (i.e., BTEX), which are present in low percentages in crude oil and, at sufficient doses, have been associated with adverse human health effects (ATSDR, 2000, 2007a, 2007b, 2010; Osborn et al., 2011). Opportunities for surface spills and leaks of BTEX-containing liquids include lined holding ponds, which are often constructed at well sites for temporary storage of “flowback” or “produced water,” which is the water that comes to the surface with the oil and gas following the hydraulic fracturing procedure. These ponds typically consist of a mixture of gas, oil, metals, fracturing fluids, and possibly naturally occurring radioactive materials (NORM) and can potentially leach into the groundwater through failures in the lining (Gregory et al., 2011; Smith, 1992). Tank battery systems, which are a group of tanks used for storing produced water and crude oil in various stages of separation, can contribute to leaks and spills. Moreover, production facilities are

sources of hydrocarbons in the refining process. Combinations of these types of facilities are found at most well sites. Although there are many different combinations of chemicals and waste products associated with hydraulic fracturing activities and therefore potentially stored at the well site, we limited our analysis to data that were publically available for review (i.e., BTEX) and regulated by the National Drinking Water maximum contaminant level (MCL), such as benzene (5 ppb), toluene (1000 ppb), ethylbenzene (700 ppb), and xylene (10,000 ppb), respectively (Colborn et al., 2011; HDR, 2011).

We performed a search for publicly available data regarding groundwater contamination from spills at U.S. drilling sites. The Colorado Oil and Gas Conservation Commission (COGCC) database was selected for further analysis because it was the most detailed. In addition, numerous articles have been published in the Colorado news media that suggest that surface spills at drilling sites in Weld County, Colorado were associated with the release of benzene at concentrations markedly exceeding state water quality standards (5 ppb) (Finley, 2011). Weld County is located on the eastern plains of Colorado and the county overlays part of the Niobrara Shale formation within the Denver–Julesburg Basin. The eastern plains have very little surface water and therefore groundwater is the main source of water supply for users in the area (Colorado Division of Water Resources, 2012; Pielou, 1998). We chose to focus on Weld County because nearly all active wells in Colorado have used hydraulic fracturing for completion, because it is the most densely populated county for drilling in the United States, and because some areas of Weld County may have a very shallow depth to water table (COGCC, 2012; Wockner, 2012; STRONGER, 2011). Given the increased attention to surface spills of benzene in the Colorado local news and the limited attention in the scientific literature given to surface activities, we investigated operator reports of groundwater contamination with BTEX at drilling sites in Weld County, Colorado, between July 1, 2010, and July 1, 2011.

To evaluate the potential impact to groundwater from BTEX in surface spills reported during our study period, (COGCC, 2011e), we specifically focused on initial measurements taken before or early in the remediation process so that we could characterize the high end of BTEX contamination that may have occurred during the course of these spills. In addition, we analyzed various other spill metrics including spill frequency, average spill size and depth, and recorded cause of the spills, as well as the fraction of spills for which remediation had been successfully completed.

Methods

COGCC database

We analyzed publically available data reported by operators to COGCC. We considered other datasets by searching multiple websites including those associated with the Wyoming Oil & Gas Conservation Commission, Pennsylvania Independent Oil and Gas Association, Pennsylvania Department of Environmental Protection: Oil & Gas Reporting Website, Texas Oil & Gas Association, Railroad Commission of Texas, and New

Mexico Energy, Minerals and Natural Resources Department Oil Conservation Division, as well as the oil and gas conservation commissions of Oklahoma, Kansas, Montana, Arizona, Idaho, Nebraska, Iowa, Missouri, North Dakota, South Dakota, and Arkansas. We chose the COGCC database because it had the most robust data set regarding surface spills.

We analyzed surface spills in Weld County between July 1, 2010, and July 1, 2011, using data reported to COGCC (COGCC, 2011e). The COGCC data were not in a tabulated or compiled format; rather, the information for each spill was found on one or more separate documents. Thus, as a first step, it was necessary to manually extract all of the relevant data and compile them in a format that was useful for analysis. The study period was selected to provide a snapshot of surface spills that were reported to have groundwater impact. According to the rules outlined by the COGCC, surface spills that are greater than five barrels in size or that impact state water sources must be self-reported by the operators. Operators are required to map the area affected by the spill, including the directional flow of the groundwater, to describe how the spill was excavated, and to submit a groundwater sampling plan to determine the extent of the groundwater contamination (COGCC, 2011a). According to the COGCC Rule 900 Series, “samples shall be collected from areas most likely to have been impacted, downgradient or in the middle of excavated areas. The number and location of samples shall be appropriate to determine the horizontal and vertical extent of the impact.” Groundwater samples were collected by various methods, including bore holes or excavation of the soil at the spill site.

Once collected, groundwater samples were analyzed for BTEX concentrations by an independent laboratory using U.S. EPA Method 8260B (COGCC, 2011d). Information regarding the spill volume, the area and depth of the spill, the type of facility from which the spill originated, and the reported cause of the spill was also extracted from the COGCC database.

BTEX concentrations

We sought to characterize BTEX groundwater concentrations during the course of the spill and the remediation, (i.e., groundwater samples that were taken early in each spill, either before or shortly after remediation began). Seventy-seven spills impacting groundwater were reported to COGCC by operators in Weld County during the study period. Sixty-two of the spill reports were accompanied by analytical BTEX concentrations from initial groundwater sampling. For 10 of the remaining spills, groundwater monitoring data were not collected during the initial stages of the spill and therefore were not used in our analysis. For the remaining five reported spills, there were no BTEX measurements available for review.

Statistical analysis

Among the 62 spills for which groundwater sampling data were available, there were in total 218 groundwater samples collected. Descriptive statistics were performed for all 218 samples pooled together and for various subsets of these data. Because there were a high number of samples below the reporting limit, PROUCL 4.0 was used to estimate means using the

Kaplan–Meier (KM) method, which is useful for analyzing left-censored data sets with multiple reporting limits and is not based on an underlying distribution of the data set (Helsel, 2005). Also using PROUCL 4.0, the 97.5% upper confidence limits (UCL) on the means were calculated using the Chebyshev inequality with KM. The 97.5% UCL was calculated rather than a 95% UCL because of the sample size, skewness of the data, and percent of samples below the reporting limit (Singh et al., 2006). Pairwise comparisons between means were evaluated using the Gehan method, a nonparametric test that is useful for censored data sets with multiple reporting limits (Millard and Deverel, 1988; Palachek et al., 1993). For the data shown in Supplemental Figure S1, nondetect values were treated as one-half the reporting limit because there were frequently too few samples per spill to permit use of the Kaplan–Meier method when estimating the mean for each spill.

Disposition of spills

We performed a follow-up survey of the remediation status of the 77 spills with groundwater impact by reviewing publicly available documents on the COGCC website and noting which spills were deemed “resolved” by COGCC such that no additional remediation was required (see Supplemental Table S1) (COGCC, Form 19A).

Results

Frequency of spills

Between July 1, 2010, and July 1, 2011, operators drilling for gas and oil in Weld County reported 77 surface spills with groundwater impact. During this time period, there were nearly 18,000 active wells in Weld County (COGCC, 2012). These findings indicated that less than 0.5% of these active wells experienced a spill that impacted groundwater. Analysis of surface spills without groundwater impact was outside the scope of the current study.

Adherence to Colorado regulations may be a contributing factor to the low percentage of surface spills with groundwater impact at active well sites. There are a number of regulations and contingency plans in place that operators must follow in order to control fluids used and stored at the surface, as well as to manage risk of groundwater contamination from surface spills should they occur. For example, the COGCC site selection criteria take into account operating near surface water supply areas, equipment to be used, secondary containment, baseline groundwater sampling, and an emergency plan (COGCC, 2011b). Placement and protection of tanks as well as industry standards for tank construction, maintenance, operation, and labeling are also regulated under Colorado guidelines. Colorado rules dictate the operating standards for permitting requirements, and for the construction and protection of holding ponds (COGCC, 2011c, 2011d; STRONGER, 2011).

Size and depth of surface spills

If known, operators reported the volume of oil or produced water spilled at a well site. Only 13 of 77 reported spills indicated a

specific volume of oil spilled (average: 24 barrels; range: 1–177 barrels). Of the 13 reported oil spill volumes, only one operator indicated that all of the oil spilled was recovered. Six reports indicated that no oil was recovered and six reports indicated that some oil was recovered (42–84%). For eight spills, the operators indicated that no oil was spilled. Regarding the remaining spills, the volume of spilled oil was noted to be unknown or to be determined.

In addition to the oil spills, operators in Weld County reported spills composed of produced water. Only 5 of 77 operators reported a specific volume of produced water spilled (average: 7 barrels; range: 1–28 barrels). Of the five reports that specified a spill volume for produced water, one operator indicated that all of the produced water was recovered. Two operators reported that no produced water was recovered from the spill and two additional operators indicated that some produced water was recovered (50–96%). For 23 spills, the operators indicated that no produced water was spilled. Regarding the remaining spills, the volume of produced water spilled was noted to be unknown or to be determined. The unknown volumes were likely due to difficulties in estimating the amount of oil or produced water that was spilled after it had saturated the ground.

Spill areas were estimated were based on rectangular dimensions reported by the operators for 59 of 77 spills. The average estimated area was 2120 square feet (range: 96 to 10,500 square feet). In 55 of 77 of the spills, the depth was also recorded and ranged from 2 to 18 feet (average 7 feet). It should be noted that these area values are probably overestimated, as fluid spills are unlikely to completely fill the rectangular dimensions.

Origin of spills

The types of facilities from which surface spills were reported to occur and the number of spills associated with each facility type are summarized in Table 1. The tank battery systems (34/77 spills) and production facilities (29/77 spills) were by far the largest sources of surface spills with groundwater impact. The remaining facilities and equipment were each reported for 5 or fewer of the 77 spills. Four of these remaining facility types, with one spill attributed to each, were associated with the tank batteries, and thus might be more appropriately counted as part of that category.

A tank battery usually provides storage for the collected oil and equipment for separating the oil from produced water (COGCC, 2011a). The tanks are commonly connected by manifolds and other piping to permit transfer of liquids from one tank to another. Production facilities are used to remove water, gases, and other impurities from the oil and natural gas. The U.S. EPA requires that secondary containment structures for tank batteries and production facilities such as dikes, berms, and other barriers be used around these two systems to help prevent migration of leaks or spills (U.S. EPA, 2009). In total, 26 of 77 spills in Weld County were retained within a constructed containment, although the spill report still indicated an impact to the groundwater. For the remaining 51 spills, the spilled fluid was not contained. The reason for failure of a required secondary containment system around tank batteries and production facilities

Table 1. Type of facility associated with groundwater impact in Weld County, Colorado, between July 1, 2010, and July 1, 2011

Facility type	Number of spills		Total
	BTEX data not available	BTEX data available	
Tank battery	5	29	34
Production facility	2	27	29
Flow line	1	4	5
Compressor station	2	0	2
Tank battery, flow line	0	1	1
Tank battery cement water pit	1	0	1
Tank battery, water tank	1	0	1
Tank battery dump line	1	0	1
Gathering line	0	1	1
Oil dump line	1	0	1
No facility type reported	1	0	1
Total	15	62	77

in Weld County was unclear. It is of note that none of the spills in Weld County was reported to be associated with a holding pond.

Causes of spills

Operators are also required to indicate the cause of the spill. Therefore, we categorized the surface spills with groundwater impact according to reported cause of the spill (Table 2). Equipment failure (47/77 spills) was the most common cause of groundwater impact, whereas 10 of 77 spills reportedly resulted from corrosion/equipment failure. Historical impact (i.e., discovery of a spill during inspection) was cited as the

Table 2. Cause of spill associated with groundwater impact in Weld County, Colorado, between July 1, 2010, and July 1, 2011

Failure Cause	Number of spills		Total
	BTEX data not available	BTEX data available	
Equipment failure	10	37	47
Corrosion/equipment failure	1	9	10
Historical impact	1	14	15
Human error	1	2	3
Multiple leaks in dump line system	1	0	1
Unknown	1	0	1
Sum	15	62	77

cause of the spill in 15 of 77 reported spills. Only 3 of 77 spills were associated with human error.

BTEX measurements

Although BTEX measurements were taken throughout the spill remediation process, we focused our analysis on BTEX measurements from groundwater samples that were taken either before or shortly after remediation began, as opposed to during the monitoring stages of a Remediation Work Plan. This allowed us to characterize the high end of BTEX contamination that occurred during the course of the spill and the ensuing remediation. BTEX data were available for 62 of 77 spills, constituting a total of 218 total samples per chemical. Summary statistics for these data are presented in Table 3. In addition, the average, minimum, and maximum concentrations of each BTEX chemical for each reported spill are illustrated in Figure S1 of the supplemental material.

As noted in Table 3, BTEX measurements for 78 of 218 groundwater samples were taken for a single spill (#2608769); thus, we considered this spill separately so that the analysis would not be overly influenced by the results of a single spill. It was not clear from the information available why so many groundwater samples were collected for this single spill.

Since we expected that the groundwater samples taken from inside of the spill excavation areas would have higher BTEX concentrations than the samples taken outside of the excavation areas, these groups of groundwater samples were analyzed separately (Table 3). In accordance with our expectations, groundwater samples collected within the excavation area had reported mean BTEX measurements that were 2.2-, 3.3-, 1.8-, and 3.5-fold higher, respectively, than groundwater samples collected just outside the excavation area. The difference in KM means from samples inside versus outside the excavation area was found to be significant for each BTEX chemical ($p < 0.05$, Gehan test).

Some groundwater sampling from reported surface spills during the initial sampling stage were done on multiple days. We hypothesized that groundwater samples taken on later dates of a spill would be associated with lower BTEX measurements. Therefore, we also analyzed the BTEX concentrations from recurring sampling events (i.e., the first, second, or third or later groundwater sampling date). As expected, the mean BTEX values decreased from the first sampling date to the second, as well as from the second sampling date to the subsequent sampling dates. The mean BTEX concentrations from groundwater samples taken during the third or later sampling date decreased 41-, 65-, 36-, and 42-fold, respectively, compared to samples taken on the first sampling date. For each BTEX chemical, comparisons of the KM means between the different sampling dates were significant, except for benzene on the second sampling date compared to the third or later sampling date ($p < 0.5$, Gehan test).

Air monitoring for BTEX has been conducted during various stages of well development and production at some well sites in Colorado (McKenzie et al., 2012). In the environment, BTEX can volatilize from soil or the water's surface, and once volatilized, BTEX disperse and readily biodegrade (e.g., benzene degrades in days, and toluene degrades in the

atmosphere within hours); BTEX can also pass through soil into the groundwater. Since BTEX are only slightly soluble in water, BTEX tend to collect at the top of the water table where they degrade more slowly than in the soil (ATSDR, 2007a). It is likely that the observed decrease in mean BTEX concentrations over the course of multiple sampling dates is, at least in part, attributable to evaporation and degradation of the BTEX chemicals.

Of the 218 measurements taken for each BTEX chemical, 60 samples per chemical were taken inside of the excavation areas during the first sampling date (Table 3). The KM mean of the 60 measurements were 1400, 2200, 190, and 2600 ppb for BTEX, respectively. These means constitute 280-, 2.2-, 0.27-, and 0.26-fold of the National Drinking Water MCLs for BTEX, respectively (HDR, 2011). Thus, the KM means for benzene and toluene in these samples were above their respective MCLs (benzene 5 ppb and toluene 1000 ppb), whereas the KM means for ethylbenzene and xylene were below their respective MCLs (ethylbenzene 700 ppb and xylene 10,000 ppb). It should be noted that the distributions of these data are highly skewed, as evidenced by the fact that the median values are much lower than the estimated means, in some cases several hundred-fold lower. None of the median values for toluene, ethylbenzene, or xylene exceed their respective MCLs.

Comparing these 60 BTEX measurements to their respective MCLs, 90, 30, 12, and 8% of the BTEX samples, respectively, were above their MCLs. These data indicate that benzene and toluene are of greater concern than ethylbenzene and xylene when considering BTEX groundwater concentrations from these surface spills. In fact, the 97.5% UCL of the mean for these 60 samples was below the MCL for ethylbenzene and xylene, and the 95th percentile measurements exceeded the MCL by only 1.3-fold for ethylbenzene and 1.2-fold for xylene.

Although the mean benzene and toluene measurements for the 60 groundwater samples taken inside the excavation areas during the first sampling date exceeded the MCL by 280- and 2.2-fold respectively, the benzene and toluene mean concentrations decreased significantly for later sampling dates and for groundwater samples collected just outside the excavation area. This suggests that actions taken by the operators to stop and remediate the spill were effective for reducing groundwater BTEX contamination. The 95th percentile toluene concentration from the 25 groundwater samples collected during the second sampling date is below the MCL, and for the third or later sampling date, none of the toluene measurements was greater than the MCL. Regarding benzene, the mean concentration decreased 41-fold in groundwater samples collected during the first sampling date compared to samples collected on the third or later sampling date. Although 59% of the benzene measurements in groundwater samples collected on the third or later sampling date still exceeded the MCL for benzene, it would be expected that benzene concentrations in groundwater samples would continue to decrease with time and as additional remediation is carried out. Likewise, the mean benzene concentration decreased two-fold in groundwater samples taken inside versus just outside of the excavation area, with only 37% of samples outside of the excavation

Table 3. BTEX concentrations (ppb) from groundwater samples taken before or early during the remediation process of Weld County surface spills involving groundwater contamination. Samples ($n = 218$) were pooled from spills that occurred between July 1, 2010, to July 1, 2011

	Count	Percent below RL ^a	50th percentile	95th percentile	KM mean ^f	97.5% UCL	Percent above MCL
Benzene							
Spill #2608769	78	88%	<1.0 ^b	14.6	6.6 ^d	24 ^d	8%
Other spills	140	27%	22	5900	920	2100	66%
-Inside excavated area	102	16%	38	6100	1100	2600	77%
-1st sampling date	60	8%	100	6100	1400	3400	90%
-2nd sampling date	25	20%	13	8900	970	5000	60%
-3rd or later sampling date	17	35%	5.5	140	34	160	59%
-Outside excavated area	38	58%	<1.0 ^b	3300	510	1900	37%
All data	218	49%	1.5	4800	590	1400	45%
Toluene							
Spill #2608769	78	100%	<1.0 ^b	<1.0 ^c	na ^e	na ^e	0%
Other spills	140	42%	2.4	8800	1200	3000	17%
-Inside excavated area	102	31%	10	10,000	1400	3800	19%
-1st sampling date	60	25%	64	10,000	2200	5800	30%
-2nd sampling date	25	36%	7.0	630	680	4700	4%
-3rd or later sampling date	17	47%	1.3	120	34 ^d	240 ^d	0%
-Outside excavated area	38	71%	<1.0 ^b	3200	430	1700	13%
All data	218	63%	<1.0 ^b	4100	750	1900	11%
Ethylbenzene							
Spill #2608769	78	91%	<1.0 ^b	49	8.2 ^d	36 ^d	0%
Other spills	140	41%	3.0	720	100	230	6%
-Inside excavated area	102	32%	4.3	780	120	290	7%
-1st sampling date	60	18%	47	900	190	460	12%
-2nd sampling date	25	32%	2.3	150	20	88	0%
-3rd or later sampling date	17	82%	<1.0 ^b	26	5.3 ^d	28 ^d	0%
-Outside excavated area	38	66%	<1.0 ^b	420	65	220	3%
All data	218	59%	<1.0 ^b	420	67	150	4%
Xylene							
Spill #2608769	78	86%	3.0	1700	230	810	0%
Other spills	140	25%	66	8400	1500	3900	4%
-Inside excavated area	102	15%	130	12,000	1800	5000	6%
-1st sampling date	60	7%	320	12,000	2600	7600	8%
-2nd sampling date	25	20%	41	6900	1100	6000	4%
-3rd or later sampling date	17	35%	7.6	390	62	310	0%
-Outside excavated area	38	53%	<1.0 ^b	2200	520	2000	0%
All data	218	47%	4.4	5600	1000	2600	3%

^aThe reporting limit was 1 ppb for all benzene, toluene, and ethylbenzene samples that were below the reporting limit. The average reporting limit and standard deviation for the xylene samples that were below the reporting limit was 2.3 ± 0.9 ppb. ^bMore than 50% of the data were below the reporting limit (RL), and therefore the reported 50th percentiles are based on nondetect values. ^cMore than 95% of the data were below the reporting limit (RL), and therefore the reported 95th percentile is based on nondetect values. ^dThese values were calculated based on fewer than 10 values above the reporting limit (9 for benzene and toluene, 7 for ethylbenzene spill #2608769, and 3 for ethylbenzene day 3 or later), and thus may not be as reliable as the other reported values. ^eIt was not possible to calculate a value because there were no measurements above the reporting limit. ^fThe Gehan test was used to test for significant differences between the following measurements: 1st sampling date vs. 2nd sampling date, 1st sampling date vs. 3rd or later sampling date, 2nd sampling date vs. 3rd or later sampling date, and inside vs. outside the excavation area. All pairwise comparisons were found to be significant ($p < 0.05$) except for benzene 2nd sampling date vs. 3rd or later sampling date.

area exceeding the MCL for benzene. This highlights the fact that benzene groundwater concentrations decrease rapidly for locations further away from the spill site.

Disposition of spills

In addition to BTEX measurements in groundwater, we also assessed the remediation status of the spills. The remediation or “resolution” process outlined in the COGCC database appeared to vary as a function of the initial BTEX concentrations. When the initial sampling was below the National Drinking Water MCLs, the spill was considered “resolved” according to the COGCC and no further remediation was required. Alternatively, if the BTEX concentrations in the groundwater from the initial sampling exceed the applicable National Drinking Water MCLs, operators are required to remove the contaminated soil and groundwater, to dispose of the contaminated waste in a state authorized hazardous waste disposal site, and to complete a Remediation Work Plan (COGCC, 2011f). As part of the remediation plan, groundwater monitoring is carried out by an independent reclamation company under the guidance of the operator. Specifically, the monitoring guidelines set forth by COGCC for remediation of groundwater indicate that a spill may be considered resolved when the measured groundwater contaminant concentrations fall below the respective MCLs for four consecutive sampling periods following excavation of the spill (COGCC, 2009). The COGCC’s use of the term “resolved” refers to this specific metric and does not refer to evaluation or resolution of other spill metrics.

For each of the 77 reported spills in Weld County with impact to groundwater, we determined whether the spill had been labeled “resolved” by COGCC as of May 2012 by accessing publically available data on the COGCC website. For 54 of 77 reported spills, resolution acceptable to COGCC was achieved after the initial excavation was performed and the operator completed a remediation plan for the spill. Alternatively, for 11 of 77 spills, resolution was achieved when a COGCC agent determined that the initial excavation of the spill and the respective BTEX analysis indicated that no further action was necessary. For all of these 11 spills, the reported initial BTEX concentrations in the associated groundwater samples were below the reporting limit following excavation of the spill. Regarding the remaining 12 reported spills, three spills were still in the process of remediation and remained unresolved per COGCC. Finally, for 9 of 77 spills no information regarding spill resolution was available for review.

To our knowledge, our analysis is the first attempt to quantitatively analyze the potential impact to groundwater from surface spills containing BTEX concentration at drilling sites where hydraulic fracturing occurs. However, there are several limitations to our analysis.

Limitations of analysis

First, our analysis was constrained by the availability and accuracy of the information on the COGCC website. All data were obtained from operator-reported spills, and therefore it is possible that additional spills may have occurred which went

unreported. In addition, BTEX measurements from groundwater samples were not available for all reported spills. However, since BTEX data were available for over 80% of the spills, and remediation status could be determined for 88% of the spills, we do not anticipate that our conclusions would change markedly if the missing data were made available. In turn, it is important to note that our findings are specific to Weld County, Colorado, and do not necessarily represent other geographical areas where hydraulic fracturing activities are conducted. Regional differences including different regulations and average depth to water table would likely impact that rate of groundwater BTEX contamination from surface spills. Since depth to the water table in some parts of Weld County is shallow, groundwater contamination from spills in this county may be more likely should a spill occur.

Another important piece of information that was not available for our analysis was baseline sampling measurements of BTEX in the groundwater. Although such sampling may have occurred, we were unable to locate it in the COGCC database. BTEX are found at low levels in crude oil and are also natural compounds found in coal and gas deposits. As such, BTEX may naturally be present at low concentrations in groundwater located in the vicinity of these types of deposits. Without baseline water quality analysis performed prior to development of the drilling site and/or prior to the spill, the background BTEX concentrations in the groundwater are uncertain.

It is possible that BTEX groundwater concentrations prior to the first sampling date may have exceeded the reported measurements because there was often a delay between the reported spill date and the first day of sampling. In addition, 15 of the spills were noted to be historical, which indicates that they were leaking for some time before the spill was discovered.

Although our study demonstrated that groundwater BTEX concentrations decreased rapidly with distance from spill site and with time after remediation began, it was beyond the scope of our analysis to estimate BTEX concentrations at any downstream receptor location for any time during the course of these spills or during the remediation process. Such an analysis may be useful in some circumstances depending on the proximity of downstream receptors of concern and the BTEX concentrations in groundwater samples taken at the spill site. In addition to potential groundwater impacts, spills of crude oil and produced water also pose potential impacts from BTEX inhalation or dermal absorption by workers or others in the vicinity of the spills. Analysis of potential BTEX exposures via these routes was also beyond the scope of this study.

Conclusions and Recommendations

Our analysis indicates that surface spills of produced water from the fracturing process or crude oil from fractured wells could pose the potential for release of BTEX chemicals in excess of the national MCLs for each compound. However, the spill reports posted on the COGCC website for Weld County, Colorado, appear to indicate that the remediation process set forth by COGCC Rule 900 Series and implemented by operators has been effective at resolving spills according to these

requirements. While there are limitations to the approach we used in our analysis, these data are nonetheless valuable in beginning to obtain a quantitative characterization of the presence and fate of BTEX in surface spills at drill sites where hydraulic fracturing activities are conducted. It also appears possible that some members of the public may hold a negative view of hydraulic fracturing activities in their community because of the lack of information regarding processes such as this one to address surface spills. It is also unclear how the determination of spill “resolution” as defined by COGCC affected any actual impacts to groundwater. As a result, we offer the following recommendations going forward in order to help address any potential impact of BTEX chemicals in surface spills to the groundwater in Weld County and elsewhere.

Recommendations going forward

It has been estimated that over the next 20 to 30 years, the density of well sites will increase in the most productive areas of oil and gas recovery (Kennedy, 2007; Pelley, 2003). The current expansion has already introduced oil and gas recovery operations to suburban and urban populations, and concerns have arisen regarding potential adverse health impacts, property damage, and ecological damage. Based on our analysis, we suggest the following recommendations, some of which are specific to the potential for BTEX groundwater contamination from surface spills, and many of which are more general and apply to multiple facets of hydraulic fracturing activities.

- (1) A comprehensive chemical risk analysis should be conducted by well operators in order to provide a formalized method for objectively identifying and evaluating the hazard and exposure potential posed by specific chemicals and chemical mixtures that are used in the hydraulic fracturing process (Panko and Hitchcock, 2011). This process, which has been referred to as chemical footprinting, can provide operators with an evaluation of positive and negative environmental characteristics including biopersistence, bioaccumulation potential, mobility, and exposure potential by multiple routes. With this knowledge, operators may better identify areas for improvement and safeguard against current and future regulatory compliance and public perception issues.
- (2) After identifying which chemicals may pose a greater risk, operators may choose to employ alternative chemicals or to implement enhanced safety measures such as additional or increased monitoring for certain chemicals on a regular basis.
- (3) Any environmental sampling plan should take into account spatial and temporal variability of chemical concentrations, achieve adequate detection limits, and properly characterize baseline or background levels of chemicals of interest. According to the COGCC website, a water sampling plan is currently under review.
- (4) Important factors such as variations in the depth of the water table on the eastern plains of Colorado should be carefully considered when evaluating the location of drilling site operations. As such, consideration should be made regarding placement of storage tanks and production facilities since our data indicated that these facilities were the most

common sources of high concentrations of BTEX in surface spills with groundwater impact.

- (5) Given the finding that many spills reported to COGCC were the result of equipment failure rather than operator error, equipment safety systems on the surface at drilling site should be carefully considered and enhanced where needed. With the remote location of many of the drilling sites in Weld County and the absence of on-site personnel to continuously monitor each well, the improvement of remote monitoring capabilities and an increase in the redundancy of spill prevention measures may be warranted at some drilling locations.
- (6) Well operators or third parties should actively engage in public education in local communities regarding the procedures used in hydraulic fracturing and horizontal drilling. Expanding and improving the public sharing of such information would be helpful in assisting workers and community members in the evaluation of personal risk versus community benefit.
- (7) Well operators should more effectively communicate the health and environmental protection procedures that they have in place prior to production so that workers and local communities are aware of the extent to which hydraulic fracturing activities may or may not pose a risk.

While our recommendations may already be fully implemented by some well operations, based on the concerns voiced in Colorado by the media and the public, it appears that the procedures for prevention and mitigation of risks associated with surface spills at active well sites are not yet fully and clearly communicated.

Acknowledgment

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Indications of Transformation Products from Hydraulic Fracturing Additives in Shale-Gas Wastewater

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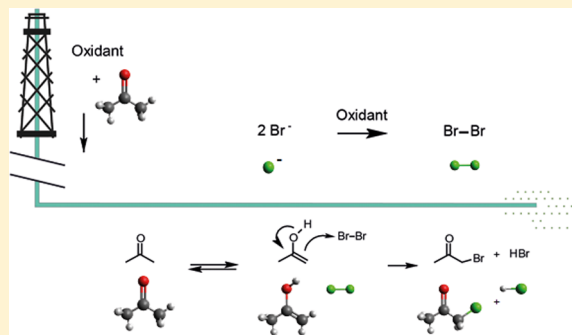
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S Supporting Information

ABSTRACT: Unconventional natural gas development (UNGD) generates large volumes of wastewater, the detailed composition of which must be known for adequate risk assessment and treatment. In particular, transformation products of geogenic compounds and disclosed additives have not been described. This study investigated six Fayetteville Shale wastewater samples for organic composition using a suite of one- and two-dimensional gas chromatographic techniques to capture a broad distribution of chemical structures. Following the application of strict compound-identification-confidence criteria, we classified compounds according to their putative origin. Samples displayed distinct chemical distributions composed of typical geogenic substances (hydrocarbons and hopane biomarkers), disclosed UNGD additives (e.g., hydrocarbons, phthalates such as diisobutyl phthalate, and radical initiators such as azobis(isobutyronitrile)), and undisclosed compounds (e.g., halogenated hydrocarbons, such as 2-bromohexane or 4-bromoheptane). Undisclosed chloromethyl alkanoates (chloromethyl propanoate, pentanoate, and octanoate) were identified as potential delayed acids (i.e., those that release acidic moieties only after hydrolytic cleavage, the rate of which could be potentially controlled), suggesting they were deliberately introduced to react in the subsurface. In contrast, the identification of halogenated methanes and acetones suggested that those compounds were formed as unintended byproducts. Our study highlights the possibility that UNGD operations generate transformation products and underscores the value of disclosing additives injected into the subsurface.



INTRODUCTION

The recent growth in unconventional natural gas development (UNGD) has led to a dramatic increase in related wastewater volumes,^{1–3} collectively referred to as flowback and produced waters. For instance, residual fluids from UNGD totaled 570 million L in 2015's first three quarters in Pennsylvania alone.⁴ Field studies have provided preliminary evidence that current wastewater-treatment practices are not sufficient,^{3,5} and risks to human and ecosystem health are inadequately explored. Furthermore, UNGD-related substances may serve as molecular markers of hydraulic fracturing activities. As a result, much interest is directed at identifying these indicator compounds, recognizing chemicals of particular concern, and considering implications for their adequate disposal.

While flowback fluids and produced water have been analyzed with regard to inorganic composition, such as halides, alkali earth ions, radioactive species, and heavy metals,^{6–9} a similar description for organic compounds is only starting to emerge. Several studies have deployed liquid chromatography (LC) with high-resolution mass spectrometry (HRMS) to the study of flowback and produced waters,^{10–14} a technique that targets roughly 90% of the disclosed chemical additives.³ However, the majority of geogenic compounds and the remaining 10% of additives are expected to be amenable to gas chromatography

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(GC) rather than LC. Available GC studies differ in (a) the level of detail aimed at identifying specific chemical substances, (b) the target-compound range and resolving power of the analytical methods, and (c) the confidence criteria applied to uniquely identify substances of interest. In all of the studies that detected substances of anthropogenic origin, such as phenols, phthalates, or biocides, investigations relied on one-dimensional gas chromatography–quadrupole mass spectrometry (GC–QMS).^{12,15–17} Although useful for many applications, QMS is not ideal for nontarget analysis due to its relatively poor mass resolution and slow acquisition time. However, it can be a useful screening tool due to the vast NIST library available. For instance, compound identifications in prior flowback- and produced-water studies^{12,15–17} were based on the similarity of mass spectra with NIST library matches. These postulated structural assignments could benefit from the application of additional confidence criteria (e.g., the use of authentic standard retention times or predictions thereof or measured retention indices such as the NIST retention index database),^{18,19} which were not used in most cases. In other studies,^{20–22} the use of comprehensive two-dimensional gas chromatography coupled to time-of-flight MS (GC×GC–TOF-MS) offered enhanced chromatographic and mass resolution. However, even the most robust GC×GC–MS study to date²⁰ grouped identifications according to substance class (e.g., PAHs and aromatics) rather than confirming them as individual compounds. In the absence of such confirmations, we note that enhanced identifications should be possible because retention-index databases, such as the Kovats measured and predicted retention indices, are available from NIST for traditional 1D GC. Lastly, particular substances of anthropogenic origin were not delineated in prior studies.²⁰ In contrast, two recent studies^{21,22} confirmed the chemical identity for single compounds using authentic standards for known UNGD additives (e.g., 2-butoxyethanol and bis(2-ethylhexyl)-phthalate), but these were in groundwaters with suspected hydraulic fracturing influence rather than confirmed flowback or produced waters. Perhaps most importantly, the focus of these previous studies has been on substances that are potentially applied as hydraulic fracturing additives; none have searched for compounds that may possibly be formed from such additives in subsurface transformations.

A recent review²³ brought forth the possibility of transformation on the basis of the consideration that certain compounds (e.g., strong oxidizers and breakers) are likely designed to react in the subsurface, and other compounds may undergo unintended transformations at elevated temperatures, pressure, and salinity. Since putative transformation products are not known to regulatory agencies, and perhaps even industrial operators, these compounds could be a primary source of unintended environmental impacts. Although their identification is needed, the possibility of transformation product formation has not been investigated in shale-gas wastewater samples.

In light of these knowledge gaps, the aims of this study were: (1) to investigate the organic-compound composition of shale-gas wastewater samples through the application of more stringent identification-confidence criteria, (2) to classify compounds according to their possible origin, and (3) to search for those substances not previously targeted by chemical analysis: those designed to react in the subsurface and those formed as transformation products.

■ EXPERIMENTAL SECTION

Overarching Approach. To identify volatile compounds, we relied on purge and trap (P&T) coupled to GC–flame ionization detection (FID) or GC–MS using authentic standards. Less-volatile, hydrophobic substances were targeted by liquid–liquid extraction (LLE) followed by GC–MS, GC×GC–FID, and GC×GC–TOF-MS. Following GC×GC–MS library searching, compound assignment was strengthened according to the following confidence criteria (listed in order of increasing confidence): (i) NIST library agreement with forward and reverse similarity greater than 85% (i.e., 850 out of 999), (ii) plausible retention behavior in accordance with the NIST Kovats retention indices, and (iii) confirmation with authentic standards. Furthermore, we note where a chromatographic feature was detected in multiple samples and assigned the same structural identity. On the basis of these assignments, we sought to classify each unique detection according to the putative origin of detected compounds by comparison to those commonly found in formation water (classified as being of geogenic origin) and disclosed additives (classified as explicitly disclosed UNGD additives). Compounds that were structurally similar or included in a family of disclosed additives were classified as implicitly disclosed UNGD additives (e.g., members of a group of compounds, such as “alkanes C₁₀–C₁₄”). Note that all disclosure databases were populated on a voluntary basis at the time of this data collection, but as of June 2015, toxic chemicals must be disclosed unless they are deemed as trade secrets (e.g., U.S. EPA Confidential Business Information). Finally, we sought to identify compounds that were likely degradation products (either because of their chemical structure or because of an abundance pattern that cannot be explained by geogenic occurrence) but which were *not* likely to be original additives. For these compounds, we postulate reactions by which they may form as transformation products.

Sample Collection and Storage. Arkansas Oil and Gas Commission personnel collected samples of Fayetteville Shale (1500–6500 ft below surface)²⁴ UNGD wastewater from production wells into 250-mL high-density polyethylene (HDPE) bottles in May 2012. A total of five of the samples were collected within 3 weeks of the initial fracturing event (i.e., “flowback waters”; samples A–E) and another sample was collected after approximately 50 weeks of the initial hydraulic fracturing (i.e., “produced water”; sample F). The water samples were shipped to Duke University (Durham, NC), where they were immediately transferred to precombusted, glass volatile-organic analysis (VOA) vials (acidified with 1 mL of 50% (v/v) hydrochloric acid (HCl) and kept at 4 °C until analysis by P&T–GC–FID or P&T–GC–MS) or to amber jars (without acidification and frozen until analysis by LLE and GC×GC–FID and GC×GC–TOFMS). Note that all of the extractions and analyses were conducted within 4 weeks of sample receipt except for the GC×GC analysis. Samples for GC×GC analysis were extracted in November 2012 from samples frozen in precombusted, amber glass jars and analyzed twice: once in November 2012 by 1D GC and again in October 2013 by GC×GC from preserved extracts.

Critically, we note that HDPE bottles are not ideal for any organic chemical analyses due to potential losses to the headspace and the polyethylene, in addition to HDPE acting as a potential source of organic chemicals (e.g., phthalates) to the sample. Nevertheless, because access to such samples is rare currently and the qualitative information contained therein is of

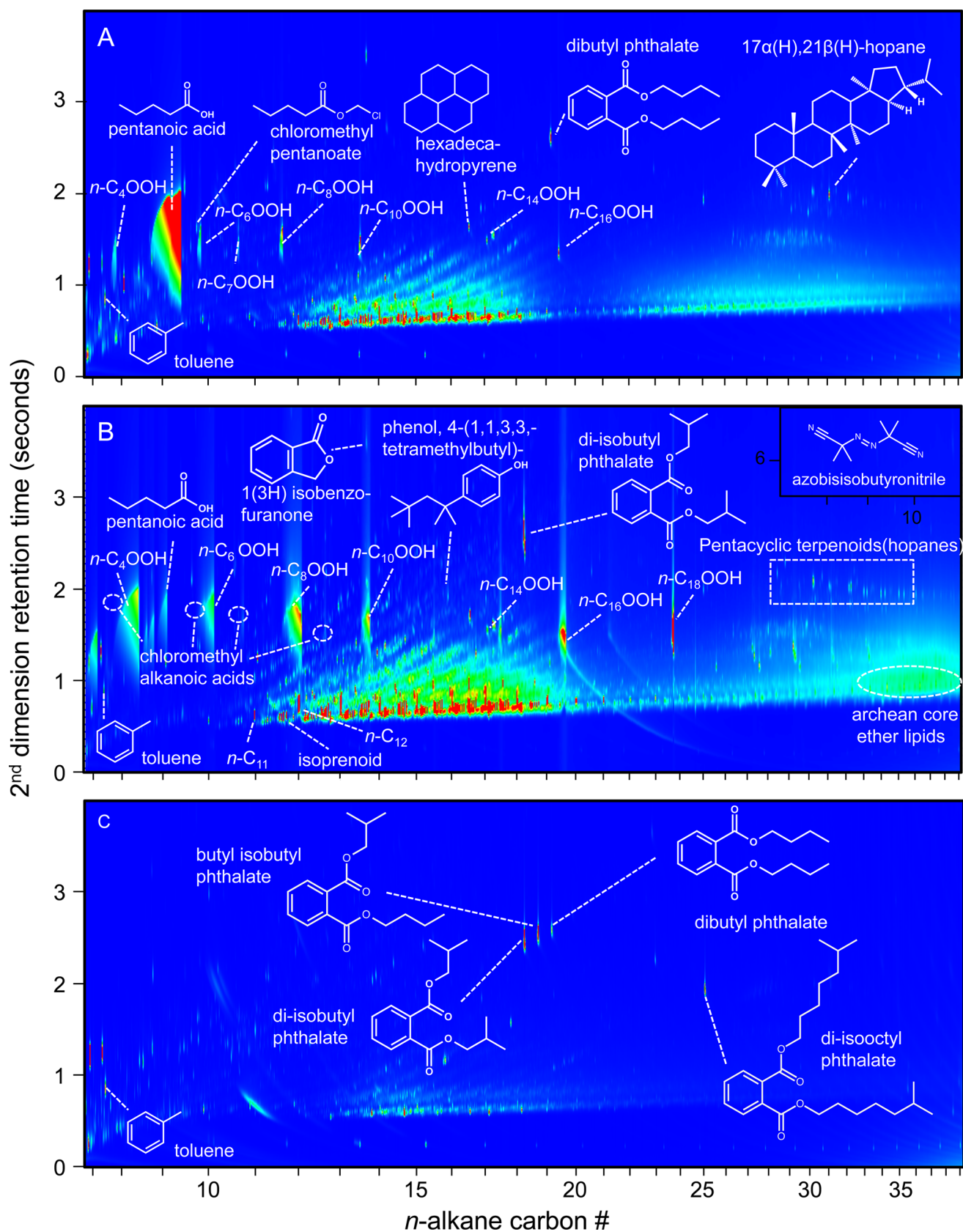


Figure 1. continued

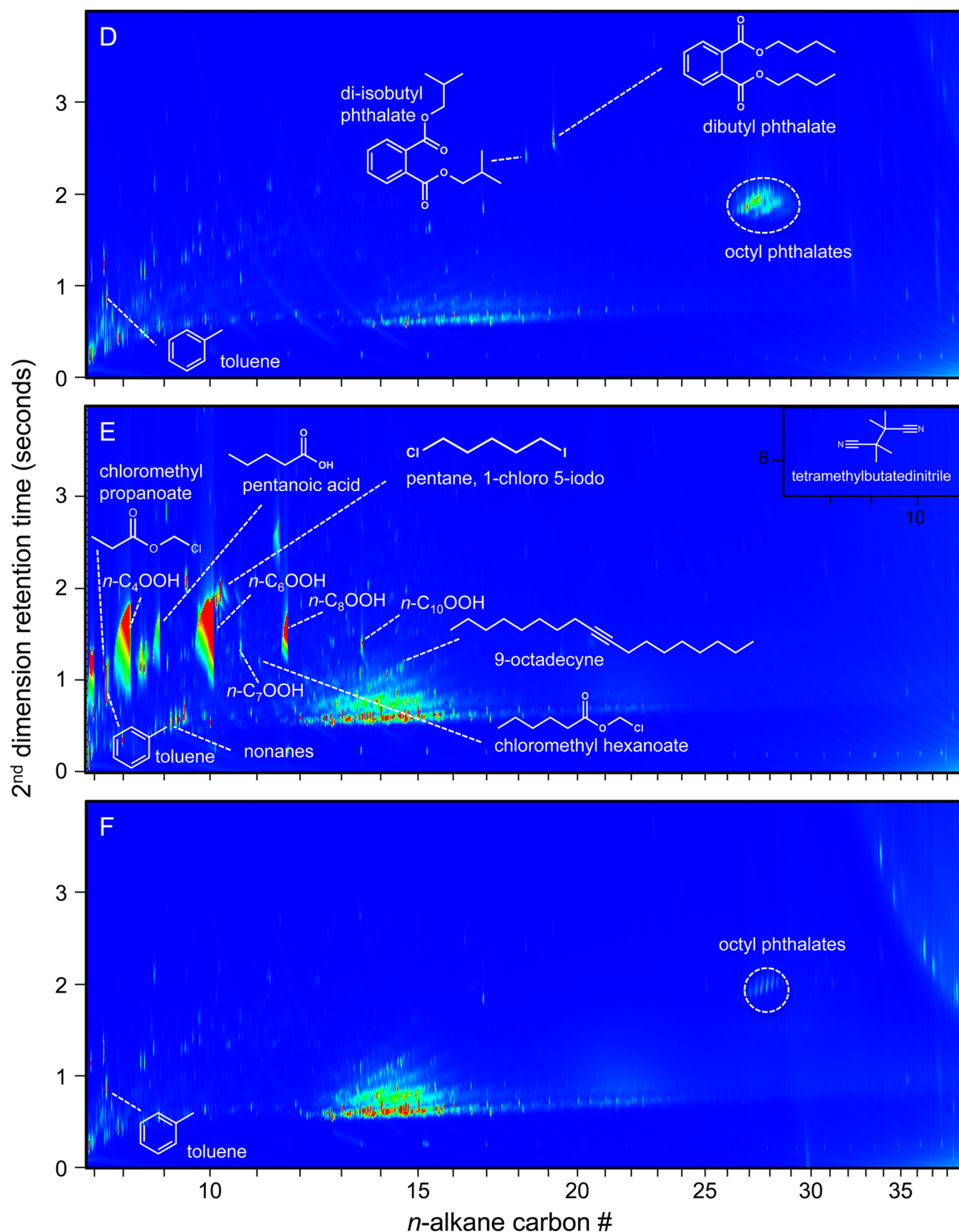


Figure 1. GC×GC–FID chromatograms for different UNGD wastewater samples with highlighted compounds and compound groups. The abscissa gives the first-dimension retention time (with respect to *n*-alkane retention time), and the ordinate displays the second-dimension retention time. The heat map reflects signal intensity, increasing from blue to yellow to red. Structures are shown for a subset of compounds identified with a high degree of confidence. Note: insets in the upper-right corners of panels display portions of the chromatograms that would otherwise be outside the selected display range. Samples A–E were collected within 3 weeks of the initial well fracture (i.e., flowback waters); sample F was collected after approximately 50 weeks of the initial hydraulic fracturing (i.e., produced water).

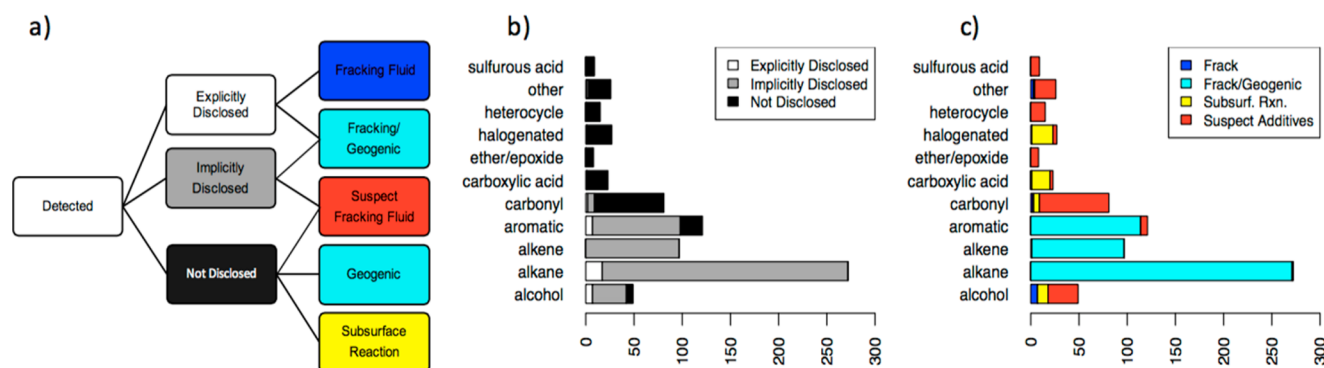


Figure 2. Compound classifications and disclosure. (a) Classification framework for detected compounds. Explicit disclosures were explicitly mentioned by chemical name or synonym on FracFocus, Skytruth, or the “Waxman List.” Implicit disclosures included structures that were nonspecific or disclosed as a group of compounds. Undisclosed items had no declarations of use in unconventional natural-gas development (UNGD) activities. Ultimate source classification was assigned using chemical structure, compound class, knowledge of geogenic materials previously reported in oil and gas plays, understanding of potential utility in an UNGD operation (i.e., suspect fracking fluid), and putative transformation pathways with likely precursors. Also shown is the breakdown of disclosure by (b) compound class and (c) source classification by compound class. A detailed list of analytes can be found in Table S1.

high value, we performed a thorough and cautious assessment for potential sample contamination (the [Control Experiments](#) section in the [Supporting Information](#)) and estimated the loss of material to the headspace (see [Table S2](#)). With up to 40% headspace, assuming equilibrium was achieved and ignoring the effect of salts and particles, the outgassing of volatile compounds may have resulted in loss of up to 15% for compounds such as benzene and toluene, less than 10% for compounds such as 1,4-dichlorobenzene, and less than 1% for ethylbenzene, xylene, toluene, naphthalene, and representative phthalates. Compounds such as octane would have been almost fully transferred to the air phase (99%), and nearly half of the octadecane (45%) would have partitioned to the air. Of course, we expect some loss of hydrophobic compounds to the HDPE to have occurred, but equilibrating into the HDPE “reservoir” would have been slow (on the order of several weeks for a compound like benzene to years for a phthalate). Therefore, using equilibrium partitioning to estimate losses could be misleading for compounds with low polyethylene diffusivities (i.e., slow transfer into the polyethylene). For example, low-density polyethylene (LDPE)-water partitioning constants (K_{IPES})²⁵ are high ($\log K_{\text{IPES}} > 5$) for many of the hydrophobic analytes in our study, and more than 99% of the material would have been lost to the HDPE bottles if equilibrium were achieved. For a more polar compound (e.g., phenol, $\log K_{\text{IPES}} = 2.4$),²⁵ 95% would be in the polyethylene (PE) with 5% in the aqueous phase and a negligible amount in the air at equilibrium. Because results indicate hydrophobic organic compounds persisted in the aqueous phase, the system was either extremely concentrated (i.e., exceeding the uptake capacity of the HDPE) or not at equilibrium. Ultimately, we caution that the results presented here are qualitative.

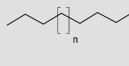
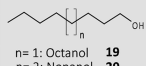
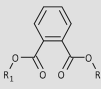
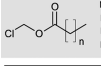
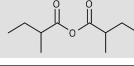
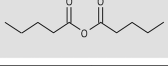
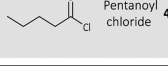
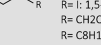
Analytical Methods. Briefly, two approaches were deployed to cover a broad physicochemical spectrum of GC-amenable organic compounds: (1) volatile compounds were analyzed by P&T–GC–FID and P&T–GC–MS (details in the [Supporting Information](#)) and (2) nonpurgeable compounds were analyzed by GC×GC–TOF–MS and GC×GC–FID (see the [Supporting Information](#) for the LLE method). These analyses were performed at the Woods Hole Oceanographic Institution on a LECO Pegasus 4D (see the [Supporting Information](#) for details).

Confidence Assignments. Traditional analytical chemistry classifies compound identifications as either tentative or

confirmed. Confirmed identifications require at least two independent pieces of evidence of a compound’s identity (e.g., mass spectral library match plus confirmed retention time with an authentic standard or confirmation of retention time against an authentic standard via the use of two distinct chromatographic columns).^{26–28} In contrast, tentative identifications require only one piece of information, and, in many published organic analyses in flowback-water literature,^{15,17,20} this has relied on GC–MS library match assignments. This reliance on single-datum compound assignments largely results because tentatively identified compounds are either not available as authentic standards or too numerous to confirm with standards within a reasonable amount of time and cost (e.g., approximately 2500 compounds at \$50 per standard would cost \$125 000). Here, we endeavor to provide confirmed identifications when possible and desirable (e.g., for “exotic” compounds beyond the standard alkanes and fatty acids through the use of available authentic standards) and provide additional confidence beyond a typical tentative identification. Several degrees of confidence were assigned (ranked from lowest to highest): (i) tentative agreement between measured and NIST library mass spectra (at least 850 forward and reverse similarity (out of 999), where 800–900 is classified as “good” and >900 is “excellent”),²⁹ at least eight coeluting apexing masses, and at least 10× signal-to-noise threshold), (ii) analyte retention index matches in the first dimension of GC×GC with a Kovats retention index library, and finally, (iii) authentic standard confirmation. Furthermore, we note where a chromatographic feature (i.e., peak at a given retention time) was assigned the same compound identity in multiple samples (i.e., convergent identifications for a given chromatographic peak).

The NIST Mass Spectral Library with Search Program (data version NIST 14; software version 2.2) was used to collect experimental and estimated retention index data for all available compounds detected in this study. Based on our own *n*-alkane standards (*n*-C₇ to *n*-C₃₆), we calculated experimental retention indices for each of the compound detections that passed the 850 forward and reverse similarity criteria. Retention indices from NIST and calculated values were both based on the “Kovats Retention Index” for temperature-programmed chromatography (see the [Supporting Information](#)), and retention agreements within ±100 were classified as positive confidence. The wide

Table 1. Overview of Selected Compounds and Compound Classes Detected in Shale-Gas Wastewater Samples

Chemical	Sample Name						analytical method	Std ³	MSL ⁴	CA ⁵	reported detection of this compound class	putative origin/reported function
Hydrocarbons (n=311)												HF additives or geogenic origin
n-alkanes	1-11, ¹ 13	1-5,7-9 11-13	3,9	7-12, 14	1,3, 5-10	3, 6-9	LLE-GC-MS GCxGC-TOF-MS	x	>850	x	Maguire–Boyle 2014, Orem 2014, Hayes 2009, Strong 2013	Geogenic origin or used as solvent/base fluid (e.g. US Pat 4739834)
BTEX	15	16-18		15			PT-GC-FID ² PT-GC-MS	x			Hayes 2009; Strong 2013; Maguire-Boyle 2014, Lester 2015, Thacker 2015	Geogenic origin or used as solvent/base fluid (e.g. US Pat 4739834)
 n = 1: Octane n = 2: Nonane n = 3: Decane n = 4: Undecane n = 5: Dodecane 1 2 3 4 5 n = 6: Tridecane n = 7: Tetradecane n = 8: Pentadecane n = 9: Hexadecane n = 10: Heptadecane 6 7 8 9 10 n = 10: Octadecane n = 11: Nonadecane n = 12: Eicosane n = 13: Heneicosane 11 12 13 14  Benzene 15  Toluene 16  Ethylbenzene 17  Xylenes (o-, m-, p-) 18												
Alcohols (n=26)												Disclosed and suspected HF additives, Transformation products
primary unsubstituted alcohols	19,21	19-21			21	22	PT-GC-MS, GCxGC-TOF-MS	(x) ⁶	>850	x	n.a.	HF additives, e.g. US 20120000708 A1
cyclic alcohols		23		23			GCxGC-TOF-MS	(x)	>850	x	n.a.	Transformation products (hydration of alkenes or ester hydrolysis) or suspect HF additives
alkyl phenols		24-27					GCxGC-TOF-MS		>850	x	Orem 2014	Biogenic or abiotic transformation products from alkylphenol polyethoxylates (see Scheme 1, C2; Stephanou & Giger, 1982)
 n = 1: Octanol n = 2: Nonanol n = 3: Decanol n = 6: Tridecanol 19 20 21 22  Benzyl Alcohol 23  Alkyl phenols R = methyl R = p-tert-butyl R = 1,1,3,3-tetramethylbutyl 24 25 26  R1 = ethyl, R2 = methyl 27												
Carboxylic acids, Anhydrides, Chlorides, Chloromethoxy esters (n= 26)												Transformation products or suspected HF additives
aliphatic monocarboxylic acids	28,29, 31,32, 34,35	28-34, 36, 37			28,32, 34		GCxGC-TOF-MS	(x)	>850	x	Orem 2007,2014, Hayes 2009	Postulated biodegradation products of geopolymeric substances (Orem); but we hypothesize they are transformation products (hydrolysis) of delayed acids
phthalates	38	39,40	38, 39	39,40			GCxGC-TOF-MS	x	>850	x	Hayes 2009, Maguire-Boyle 2014, Orem 2014, Lester 2015, Ferrer 2015, Drollette 2015	Phthalates mentioned as suitable organic acids (US Pat 20070125536) or as diverting agents (fracfocus.org, e.g. Nabors Completion and Production Services)
chloro methoxyesters	42	41,44			41,43		GCxGC-TOF-MS		>850	x	n.a.	Putative undisclosed delayed acid (Scheme 1A)
anhydrides and acyl chlorides	45,47						GCxGC-TOF-MS		>850	x	n.a.	Carboxylic acid anhydrides are used as delayed acids, e.g. US Pat 8387696. The acyl chloride is either a transformation product of an anhydride (hydrolysis) or is used itself as additive, releasing acid moieties.
 n = 2: Butanoic acid n = 3: Pentanoic acid n = 4: Hexanoic acid n = 5: Heptanoic acid n = 6: Octanoic acid 28 29 30 31 32 n = 7: Nonanoic acid n = 8: Decanoic acid n = 9: Undecanoic acid n = 10: Dodecanoic acid n = 12: Tetradecanoic acid 33 34 35 36 37  R1, R2 = butyl: Dibutyl phthalate R1, R2 = isobutyl: Diisobutyl phthalate R1, R2 = isooctyl: Disooctyl phthalate 38 39 40  n = 1: Chloromethyl propanoate n = 3: Chloromethyl pentanoate n = 4: Chloromethyl hexanoate n = 6: Chloromethyl octanoate 41 42 43 44  2-Methylbutanoic anhydride 45  Pentanoic anhydride 46  Pentanoyl chloride 47												
Halogenated compounds (n=13)												Transformation products or suspect HF additives
halogenated benzenes						56	PT-GC-FID ² PT-GC-MS	x			Hayes 2009	Other isomers were not detected, therefore unlikely a transformation product; probably biocide additive
halogenated pyran	57 ¹						GCxGC-TOF-MS		>850	x	n.a.	Electrophilic addition with epoxide intermediate (see Scheme 1, B3)
halomethanes				48		48	PT-GC-MS		930-960		Hayes 2009, Getzinger 2015, Thacker 2015	Dichloromethane mentioned on SkyTruth (function unclear); could be formed by radical substitution of methane (Scheme 1, B1) or bacteria
halogenated alkanes		58, 59			52	55, 60	GCxGC-TOF-MS		>850	x	Hayes 2009, Maguire-Boyle 2014	Transformation products formed by addition or substitution reactions (Scheme 1B). Precursor compounds: Alkanes, alkenes can be either geogenic or used as solvents (e.g. US Pat 4739834); alcohols or diols are used for scale inhibition or crosslinking (Elsner & Hoelzer)
	51, 54		51, 53	51, 53	51	51	LLE-GC-MS		830-960		n.a.	
halogenated acetones	49	49	49, 50	49, 50	49, 50		PT-GC-MS		>850		n.a.	Halogenation of carbonyl groups of acid or ketone (catalyzed by acid or base)
 Dichloromethane 48  1,4-Dichlorobenzene 56  2-Chloromethyl-(tetrahydro)pyran 57  1,2-Diiodoethane 51  2-Bromo-2-methylbutane 58  4-Bromoheptane 59  1-Iodo-2-methylundecane 60  1-Chloro-5-iodopentane 52  1,5-Diiodopentane 53  CH2CH3: 1-Iodoheptane 54 C8H16CH3: 1-Iodotetradecane 55												
Nitriles (n=2)	61 ⁷			62				(x) ⁸	>850	x	Orem 2014	Chemical transformation products of suspect HF additives: AIBN is a radical initiator and TMSN is a transformation product (Scheme 1, C1)
Other (n=39)												see Table S1

^aBold-faced numbers indicate compound identity and correspond to their respective structures in the gray boxes. ^bFID signals were below limit of quantification but above the limit of detection for several instances. ^cStd: x = confirmed with authentic standards. ^dMSL: x = Mass Spectra Library forward and reverse similarity (reported as a percent of a total possible match of 999). ^eCA: positive confidence assignment via Kovats retention-index match. ^fSome of the depicted compounds were confirmed by authentic standards. ^gCompounds 61 and 62 are displayed in Scheme 1.

tolerance threshold was chosen to allow for enough deviation from NIST database (e.g., to account for experimental and configurational variances) while still narrow enough to reject egregious identifications. These results were compared to a boiling-point-prediction model (which was less robust to the broad spectrum of compounds observed here), whose approach and results are available in the [Supporting Information](#).

■ RESULTS AND DISCUSSION

Among the six flowback- and produced-water samples, there were broad differences in the hydrocarbon chemical distribution reflected in the GC×GC chromatograms ([Figure 1](#)), indicating these may potentially serve as chemical fingerprints and carry information about the UNGD process as well as the geologic formation. Despite these differences, there was a remarkable similarity in the total number of compounds detected via GC×GC–FID (2550 ± 140 for $n = 6$; 2762, 2565, 2600, 2346, 2490, and 2523 for samples A through F, respectively). Note that not all peaks are visible in [Figure 1](#) due to scaling. Of these nearly 2500 compounds, GC×GC–TOF-MS was able to postulate identifications for 729 unique compounds (using relatively strict MS library match criteria). After the application of the Kovats retention index match to reject egregious identifications, the number of confident assignments was reduced to 404, just 55.5% of the original total number of identifications ([Table S1](#)). This is reasonable compared to the 25% false positive rate that results from MS library match only shown for much smaller data sets ($n = 30, 45$, and 87).³⁰ In the discussion that follows, we refer only to confidently identified chemicals (i.e., those that pass the relatively strict MS criteria and the Kovats retention-index match). As a consequence, tentative identifications that did not pass the retention index match were omitted from this discussion but are nevertheless listed in [Table S1](#) for completeness.

Detected Substance Classes and Disclosure Rates.

Hydrocarbons (i.e., alkanes, alkenes, and aromatic compounds) were most abundant in our detected list of compounds ([Figure 2](#)). This is consistent with previous findings^{15,16,20} and is not surprising because such hydrocarbons are (a) disclosed as additives or solvents in practically every UNGD operation²³ and (b) may stem from the geologic target formation. In contrast, substances with functional groups like carbonyl compounds, alcohols, halogenated compounds, carboxylic acids, ethers, epoxides, and others (e.g., nitriles and siloxanes; [Table 1](#)) were detected in smaller numbers. Typically, many of these compounds are not reported in shale formations^{16,20} or found in crude oil extracts, suggesting an anthropogenic origin. Such compounds could be informative because functional groups and their associated reaction chemistry indicate a putative purpose as fracking additives. Strikingly, it is precisely these compounds, those potentially performing the critical subsurface chemistry, that are disclosed at a much lower rate compared to alkanes and petroleum hydrocarbons. For example, the disclosure frequency (i.e., number of reports per number of total disclosures) on FracFocus³¹ is less than 1% for the organohalogen (other than the biocides, benzyl chloride and dichloromethane), less than 5% for carboxylic acids (other than formic acid, acetic acid, and their salts), and between 2 and 5% for ethers and epoxides.^{23,32,33} In comparison, petroleum distillates are disclosed in roughly 100% of all UNGD operations reported on FracFocus, with additional disclosure of specific aromatic structures in 30 to 50% of operations.^{23,32,33}

Sample Heterogeneity and Emerging Similarities: Insights from GC×GC. First, we note that all six of the

UNGD wastewater samples were derived from a single shale play. Although a sample set of six is small and may fail to capture the true heterogeneity of flowback and produced water, this is one of the largest data sets of its kind. Nevertheless, caution should be taken in extending these results to other produced and flowback waters, which could vary between and within a single formation, as a function of time since spud date and, of course, due to the variability in additives (i.e., fracturing-fluid composition from well-to-well). This work aims to build on the growing body of knowledge that seeks to delineate the possible chemical characters of UNGD waste fluids.

Comprehensive two-dimensional GC offers enhanced resolution of hydrophobic complex mixtures that has revolutionized the study of oil and gas extracts^{27,28,34–36} ([Figure 1](#)). Commonly, and in our GC×GC analysis, substances are separated according to vapor pressure in the first dimension (i.e., abscissa or x -axis) and according to polarity in the second dimension (i.e., ordinate or y -axis). Note that samples A–E were collected at the same well age and from the same shale play, yet each exhibits a heterogeneous chemical character visible in GC×GC space.

As expected for shale-derived samples, the majority of the components were detected between n -C₁₁ and n -C₂₀ in the first dimension. A higher percentage of lower-boiling, lower-polarity compounds (n -C₁₂ to n -C₁₆ and roughly 0.5–1 s in the second dimension) was observed in the samples E (and F, the produced water) compared to the samples A and B, which had a broader distribution in both dimensions (n -C₁₁ to n -C₂₀ and roughly 0.5–1.5 s). This difference does not correspond to weathering, in which one would expect losses of lower-boiling and higher-polarity compounds preferentially.³⁷ That is, if samples E and F were similar to A and B but had experienced some weathering event (either in the field, during transport, or in the lab), then E and F would have some loss in the “front end” (low-boiling and high-polarity compounds) but not at the “back end” (higher-boiling, lower-polarity compounds).^{37,38} Since E and F are lacking both the high- and low-volatility compounds relative to samples A and B, then the difference is likely due to authentic variations in the chemistry of the source water rather than weathering in the field or a sampling artifact. As all samples were derived from the same shale formation and are of the same age (except for the relatively older F), this suggests that some of the detected hydrocarbons may be hydraulic fracturing additives, contributing to the geogenic hydrocarbons.

Intersample differences became even more pronounced in the polar regions of the chromatograms (between 1 and 2 s in the second dimension; [Figure 1](#)). Here, carboxylic acid peaks occurred at regular intervals in a ladderlike fashion. An even-over-odd preference is visible in several samples (B, E, and A), which is consistent with Orem et al.’s reporting of C₁₂, C₁₄, and C₁₆ carboxylic acids¹⁶ as anaerobic biotic breakdown metabolites of geopolymeric substances³⁹ and is expected for mixtures of these compounds due to the biological production pathways used to make them (both in the environment and in industry).^{40–42} However, in some instances, this pattern of likely geogenic origin is overlain by an overwhelming dominance of a specific alkanolic acid (e.g., pentanoic acid in A and butanoic and hexanoic acid in E). This suggests that these fatty acids derive from UNGD additives through direct addition or through in situ production from an abundant precursor additive. For instance, in sample A, pentanoic acid occurred together with chloromethyl pentanoate, pentanoyl chloride, and pentanoic acid anhydride, whereas hexanoic acid co-occurred with chloromethylhexanoate in sample E. These chloromethyl alkanolic acids, alkanoyl

chlorides, alkanoyl anhydrides, and their potential transformation pathways are described in detail below.

A total of two classes of compounds emerged that are almost assuredly of geogenic origin: the archaic core ether lipids and the pentacyclic terpenoids (i.e., hopanes). These appear in the biomarker region ($>n\text{-C}_{25}$ and around 2 s in the second dimension) and can be used to trace or fingerprint the shale formation itself, gauge the thermal maturity of the oil hydrocarbons there, and ultimately determine the origin (e.g., kingdom of life) of the organic matter that gave rise to the oil in the source rock.^{26,28,43–45} Samples A and B showed a clearly defined hopane biomarker region (see Figure S3), and thermal maturity indicators, such as the T_s/T_m ratio (where T_s is $18\alpha(\text{H})$ -22,29,30-trisnorhopane and T_m is $17\alpha(\text{H})$ -22,29,30-trisnorhopane), suggested that samples were of the same geological age (see the Supporting Information). Note that sample A was much less concentrated than B, but the relative proportions and distribution of hopanes were similar between the two. Other samples had indiscernible levels of hopane biomarkers. Steranes, which can indicate geological formation source information, were not detected. Nevertheless, where available, these biomarkers are powerful for tracing shale wastewaters or in environmental forensics associated with such source apportionment between heterogeneous, complex mixtures.

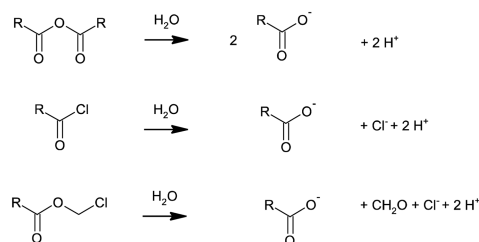
Sample heterogeneity persisted at higher second dimension retention times (2–3 s), where multiple phthalate esters were detected. Although their occurrence clearly indicates an anthropogenic influence, we caution that polymer containers utilized during the initial sample collection by the Arkansas Oil and Gas Commission raise concern as a potential source of phthalates. However, we do not consider this the prime or only source in our study for the following reasons: (a) the phthalate esters were not detected in all samples, even though all of the samples utilized the same types of containers over the same time frame; (b) the specific type of detected phthalate varied among the samples but would not have varied between the containers; and (c) laboratory control studies in which saline water was equilibrated with the containers over 120 days showed no detectable phthalates at a seven parts per billion detection limit (see the Control Experiments section). Thus, while we caution that phthalates are ubiquitous industrial chemicals (i.e., potentially derived from pipe utilized in the field), we expect that these compounds are authentic to the sample and derive from hydraulic fracturing operations. Indeed, *bis*(2-ethylhexyl) phthalate is disclosed as a diverting agent (e.g., from Nabors Completion and Production Services),⁴⁶ and *di-n*-octyl phthalate was reported in UNGD wastewater.⁴⁷ Undisclosed phthalates, such as diisobutyl, dibutyl, butylisobutyl, dioctyl, and diisooctyl phthalate, were also detected, suggesting that phthalates may have more pervasive uses in hydraulic fracturing than indicated by their disclosure rates.²³ Note that all phthalates were confirmed with authentic standards (and all but dioctyl phthalate passed the Kovats retention index confidence check).

Finally, two additional compounds at very high retention times (around 6 s; Figure 1 insets) are strongly indicative as UNGD additives: azobis(isobutyronitrile) (AIBN), a disclosed, common radical initiator,⁴⁸ in sample B; and tetramethylsuccinonitrile (TMSN), its direct transformation product, in sample E (Scheme 1C; discussion below). Radical-initiating azo compounds are occasionally reported in the “Waxman List” and on FracFocus, and they do not have geogenic origins. As such, these compounds were categorized as hydraulic fracturing additives

Scheme 1. Mechanisms of Subsurface Reaction-Product Formation^a

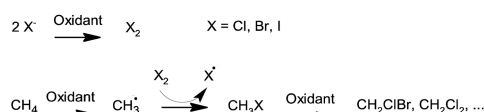
A) Putative Delayed Acids

Acid anhydrides, Acyl Chlorides, and Chloromethyl alkanooates

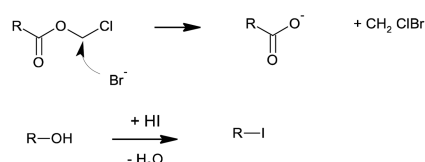


B) Putative Halogenation Reactions

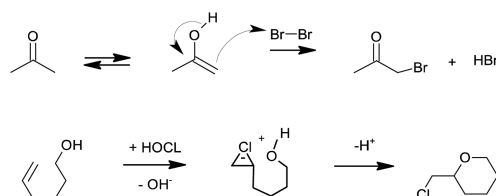
1. Radical Substitution



2. Nucleophilic Substitution

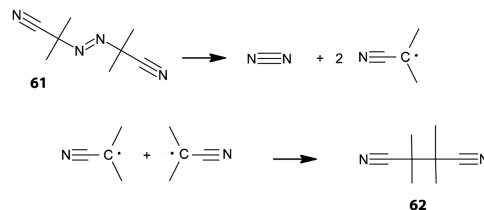


3. Electrophilic Addition

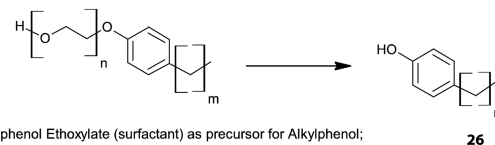


C) Transformation reactions of disclosed additives

1. Desintegration of Radical Initiator AIBN and Formation of Transformation Product TMSN



2. Degradation of Alkylphenol Ethoxylate to Alkylphenol



Alkylphenol Ethoxylate (surfactant) as precursor for Alkylphenol;
m=7 Octylphenol (Ethoxylate); m=8 Nonylphenol (Ethoxylate)

26

^a(a) Putative delayed acids render acidic protons after a hydrolysis reaction. (b) Putative halogenation reactions can occur via radical-mediated substitution, nucleophilic substitution, or electrophilic addition. (c) Demonstrated transformation pathways of disclosed hydraulic fracturing additives, such as radical initiators (AIBN (azobisisobutyronitrile) degrading to TMSN (tetramethylsuccinonitrile))⁴⁵ and alkylphenol ethoxylates degrading to alkylphenols, which can occur biotically and abiotically.

and present interesting opportunities for radical-initiated transformation pathways in the subsurface.

Structural Classification and Quantitative Overview of Detected Compounds. To provide an accessible overview, we classified compounds according to their chemical structure (Tables 1 and S1) in a similar way as presented in a recent review of disclosed UNGD additives.²³ Only those compounds that could be confirmed via more rigorous confidence assignment criteria (e.g., 311 hydrocarbons and 27 alcohols via methods such as authentic standards (“Std”), mass spectral library agreement score (“MSL”), and retention-index-confidence assignment (“CA”)) are presented in the main text (Table 1), whereas a comprehensive overview of all tentative identifications ($n = 729$) are presented in Table S1. Out of these classes, structures are given for those substances that stand out because of their occurrence in several samples (i.e., columns “A” to “F” in Table 1) and functional groups that indicate a specific reaction chemistry. Identifications are supported by CAS (Chemical Abstracts Services) numbers (where available), putative origin (Table S1), and patent number (where available), as well as by the provision of references to previous studies that tentatively detected the same chemicals in flowback waters.

Hydrocarbons were the most prominent compound class, and they are both geogenic and utilized as UNGD additives. Among the hydrocarbons, the well-known groundwater contaminants benzene, toluene, ethylbenzene, and xylenes were present in some of the samples and occurred in concentrations up to $7.3 \pm 0.5 \mu\text{g L}^{-1}$ (Tables S2 and S3), although we emphasize that up to 15% of the original benzene may have partitioned to the headspace in the nonideal collection approach (i.e., HDPE containers with headspace). If equilibrium with the HDPE were achieved (>2 weeks), functionally all of the BTEX would have partitioned into the container itself. In addition, many alcohols were detected and allocated as putative fracturing chemicals (additives) or their transformation products because (a) long-chain alcohols are occasional UNGD additives commonly used as solvents (e.g., 1-decanol, 2-ethyl-1-hexanol, and isopropyl alcohol, which has a disclosure frequency of 47–50%)^{23,33} and (b) may form by degradation from ethoxylated alcohols, either by abiotic oxidation of the weak C–H bonds next to an ether group⁴⁹ or in biotic degradation.⁵⁰ Such ethoxylated alcohols are disclosed as frequent additives (between 65 and 100%) and have been detected in flowback.⁵¹ (c) Finally, alcohols can be products of chemical hydration of alkenes or of ester hydrolysis,⁵² and certain alcohols can be the biotic fermentation product of sugars. However, because alcohols are typically not prominent in shale-gas formation water and due to the structural similarity to disclosed compounds, other detected alcohols were also considered as suspect UNGD additives or transformation products.

Remarkably, there are numerous compounds that are not likely of geogenic origin and are also *not* known reported hydraulic fracturing additives (Table 1). In particular, there was a high abundance of certain carboxylic acids (pentanoic acid in sample A and butanoic and hexanoic acid in sample E) together with the occurrence of hitherto unknown putative-fracturing additives (e.g., chloromethyl alkanooates and alkanooic anhydrides). Another example is the rather high occurrence rate of alkyl phenols and benzyl alcohol, which stands in contrast to the low frequencies at which these compounds are reported as fracturing additives on FracFocus (nonylphenol <0.2%; benzyl alcohol is not disclosed).^{23,32,33} Additionally, there is strong evidence for halogenated hydrocarbons that were observed in all

flowback- and produced-water samples. With the exception of benzyl chloride (reported on FracFocus in 6–7% of all operations^{23,32,33}), halogenated hydrocarbons are practically nonexistent in lists of reported hydraulic fracturing additives. Specifically, the brominated, iodated, and chlorinated (e.g., bromoacetone, 1-chloro-5-iodopentane, and dichloromethane) substances that were detected in our study were not disclosed as additives in fracturing applications except for the seldom-reported dichloromethane (0.01% frequency).^{23,32,33} This contrasts strongly with our finding that dichloromethane and 1-iodo-tetradecane were detected in two samples, and chloroacetone or bromoacetone were detected in five out of six samples. Considering the low disclosure rates, we entertain the hypothesis that these chemicals formed as unintended transformation products in the process of the hydraulic fracturing process. In the subsequent discussion, we postulate putative reaction mechanisms that may lead to formation of these compounds.

Proposed Reaction Mechanisms Leading to Transformation Products. In general, transformation products can arise in both abiotic and biotic reactions, and very few environments are truly sterile.⁵³ During the hydraulic fracturing process, conditions are met that are favorable for abiotic processes, such as elevated temperature, pressure, salinity, and the use of strong oxidizing agents and biocides. Although the possibility of biological transformations must not be ignored, here we consider primarily abiotic transformations to explain products hypothesized to form during the UNGD process (e.g., halogenation reactions are possible in halotolerant organisms but are generally not considered a broadly distributed, common metabolic capability).⁵⁴ In contrast, conditions at the surface for flowback and produced water are quite favorable for biogenic transformation, and we presume degradation was assuredly occurring after the fracturing process.

1. Hydrolysis Reactions of Putative Delayed Acids: Intended Transformations. Detected alkanooil anhydrides, alkanooil chlorides, and chloromethyl alkanooates provide an example of likely *intended* abiotic subsurface transformations, as they can function as delayed acids (Scheme 1). In the course of a hydraulic fracturing operation, a base fluid must first be low-friction to convey the fracturing pressure underground, and then the fluid must become viscous to effectively transport proppants into the formation, and subsequently, the fluid must become nonviscous again to facilitate flow back to the surface. To catalyze the last transition, operators add so-called “breakers” to destroy the 3D polymer structure of a water-based gel and thereby decrease its viscosity. In the case of guar gum, by far the most commonly applied gel-forming agent in UNGD operations,²³ borates are used as cross-linkers to form three-dimensional polymer structures. Here, acids serve as convenient breakers by shifting the acid–base equilibrium of borate to boric acid. This sequestration of borate ions as cross-linkers causes the 3D gel structure to break.⁵⁵ If such a strategy is pursued, the *timing* of acid addition is crucial. If cross-links were broken up too early, proppants could not be transported, and the fractures in the formation would close prior to gas recovery. For this reason, alkanooil anhydrides, alkanooil chlorides, and chloromethyl alkanooates can be attractive reagents. These first undergo chemical hydrolysis reactions and subsequently release their acid equivalents (Scheme 1A) after the appropriate delay time (i.e., they are *delayed acids*). Potentially, this time could be tuned by choosing different compounds in varying proportions and by

changing the chain length of the alkanolic acid (e.g., C₃, C₄, or C₅) to lend the additives different degrees of hydrophilicity.

2. Halogenation Reactions: Unintended Transformations. The halogenation of hydrocarbons provides a potential example of *unintended* transformation reactions that may generate problematic byproducts (Scheme 1B). Even though biotic organohalogen production, e.g., by marine algae, sponges, and bacteria is known,⁵⁶ we hypothesize that detected compounds are attributable to abiotic transformation (with the possible exception of biotic halomethane formation in the reservoir). Specifically, underlying reaction rates may be enhanced due to the elevated temperatures and high salinity prevailing in the subsurface, and many of these reactions could be triggered by the strong oxidants introduced as breakers in the course of the hydraulic fracturing process.²³ For example, in the presence of strong oxidants, halides can form molecular halogens (Cl₂, Br₂, and I₂) and, simultaneously, these oxidants can also attack methane, which is present in high concentrations in the formation (Scheme 1B1). Once methyl radicals are formed, they are readily halogenated under these conditions in radical substitution reactions. Alternatively, nucleophilic attack (S_N2) by halide ions (Cl[−] and Br[−]) may generate dihalomethanes out of the putative chloromethyl alkanolate additives, even in the absence of oxidants (Scheme 1B2). This is an example of how undisclosed additives may potentially generate unintended byproducts, underscoring the importance of disclosing UNGD additives. Finally, molecular halogens and hypohalogenic acids may also facilitate electrophilic addition reactions, which can explain the formation of halogenated acetones and a halogenated pyrane observed in this study (Scheme 1B3).

3. Transformation Reactions of Disclosed Additives: Products of Known Additives. Several compounds detected in our study may stem from transformation reactions of disclosed additives (Scheme 1C). One example is the formation of TMSN (62) from the radical initiator AIBN (61). AIBN (61) spontaneously and abiotically releases a molecule of nitrogen gas originating from the azo group to form two 2-cyanoprop-2-yl radicals,⁵⁷ which can initiate polymer chain reactions or recombine to TMSN (62). These compounds were found in two out of six samples in our Fayetteville sampling, suggesting they may serve as UNGD additives to initiate polymerization more often than reflected by the national disclosure rate (0.01%). (An alternative source could be leaching from polymers; see Elsner and Hoelzer).²³ Another example of a reaction byproduct of a known additive is benzyl alcohol (23), which is not itself reported as UNGD additive.²³ However, benzyl alcohol can form via abiotic hydrolysis of benzyl chloride in an S_N1 reaction. Indeed, benzyl chloride is a rather frequent additive (application frequency of 6%–7% on FracFocus).^{23,32,33} Finally, even though phenols are reported as naturally occurring constituents in shale formation water,^{46,58} and phenols are also occasionally reported as UNGD additives,²³ we hypothesize that at least some of the phenols detected in our study are formed (biotically or abiotically) as transformation products. In particular, (a) the structures of the compounds 4-*tert*-octyl phenol (a precursor in the synthesis of octylphenol polyethoxylates) and *p*-*tert*-butyl phenol (26, 25) appear too specialized to be of likely natural origin, and (b) the reports of phenols in UNGD databases are greatly outnumbered by the instances at which *alkoxylated* phenols are reported.²³ For this reason, we hypothesize that these phenols are formed as transformation products of the respective alkylphenol ethoxylates^{59,60} (Scheme 1C). Note that alkylphenol ethoxylates can give rise to alkylphenols as

metabolites via ω -oxidation or central fission^{50,59,61,62} or abiotic oxidation of the weak C–H bond adjacent to ether bonds.⁴⁹

Compounds outside the Confidence Assignment. The compounds discussed above passed careful evaluation of data quality by high MS library match scores (>85%), as well as additional confidence assignment criteria, making this study conservative relative to previous investigations. Had we applied less-strict proceedings, some other observations would be made, which we describe briefly. Beyond our strict confidence assignments, there were indications for more halogenated compounds. For example, 1-chloro-5-iodo-pentane was detected in five samples with at least 620 forward and reverse similarities in the MS library (and with greater than 850 similarities and passing the retention index CA in an additional sample) and iodoheptane (320 similarities) in three samples. Furthermore, a total of nine sulfurous acid alkyl esters were tentatively detected (one passed the Kovats confidence assignment: sulfurous acid, 2-ethylhexyl isohexyl ester). These are not reported as fracturing additives; only inorganic sulfite salts (paired with ammonium or organic ammonium ions) are disclosed to serve for oxygen scavenging or corrosion inhibition. Our findings might indicate their use as additives, although the purported utility of the sulfurous acid esters is unclear. Nevertheless, the detections are supported by Strong et al.,²⁰ who detected a similar sulfurous acid ester, namely sulfurous acid, dodecyl 2-propyl ester.²⁰

Implications for Monitoring and the Environment.

These data demonstrate that UNGD wastewater not only contains fracturing additives and compounds of geogenic origin but also intended and unintended transformation products generated during the process. This has the following important consequences. (1) Standard monitoring methods are not sufficient for a proper assessment of UNGD wastewaters. Regularly monitored compounds (e.g., via EPA standard methods) overlook a variety of constituents, especially transformation products. Consequently, more comprehensive monitoring concepts are needed, especially as advanced instrumentation becomes more accessible. For instance, GC×GC–TOF-MS (among other advanced techniques) allows the detection of undisclosed compounds or transformation products, which could not be observed in targeted analysis. In the absence of the broad application of advanced analytical techniques, a primary screen of diesel-range organic compounds would enable one to identify samples for which a more thorough GC×GC analysis was merited.^{3,22} Note that here, we are only describing methods for the hydrophobic organic compounds, and a comprehensive chemical description of these waters is indeed a complex undertaking (i.e., for inorganic materials, naturally occurring radioactive materials (NORMS), and polar organic analytes, which are a large fraction of the disclosed chemical database).³ In addition, as UNGD expands, heterogeneities between formation waters, injected fracturing fluids, and transformation products must be elucidated, and studies pursuant to this should be undertaken whenever possible. (2) Full disclosure of UNGD additives is needed to accurately gauge risk associated with UNGD wastewaters. Current practice (June 2015 to present) maintains that it is sufficient to disclose merely whether an additive is toxic or not while concealing the chemical identity due to its proprietary nature. Here, we show that even nontoxic precursors can be converted to problematic products, and disclosing chemical additives could enable enhanced prediction, toxicity screening (see the [Supporting Information](#) for discussion), and the mitigation of unintended byproducts. Furthermore, because waste-treatment practices tend to target

biodegradable compounds, it is possible that standard waste-treatment practices would not capture many of these non-biodegradable components. In high-salinity waters, these could go on to give rise to enhanced disinfection byproduct formation in drinking-water treatment plants whose intakes are downstream from treated-waste-receiving waters. Thus, the information presented in this study could aid in the development of targeted treatment practices that could prevent such unintended consequences. (3) We recommend iodide monitoring^{63,64} alongside chloride and bromide as well as iodated, chlorinated, and brominated compounds. This is particularly important because iodo-organics' health impacts are often more severe than those of chlorinated and brominated species,^{65,66} and it is not yet clear to what extent they are present in UNGD wastewaters. Furthermore, likely exposure routes of UNGD wastes to the environment should be evaluated and addressed if treatment is deemed necessary.^{3,64,67} Overall, these considerations illustrate the far-reaching consequences of an adequate identification of transformation products. Here, we present a path for further research in this direction, which must be accompanied by toxicological studies and studies of biological transformation pathways and ultimately channeled into strategies for wastewater treatment (see the [Supporting Information](#) for further discussion).

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.est.6b00430](https://doi.org/10.1021/acs.est.6b00430).

Additional details on analytical details, confidence assignment, control experiments, quantitative results and corrections for potential losses, hopane biomarkers, toxicology and water treatment implications, and references. Tables showing a full list of mass spectral library matching derived tentative identifications and putative compound origins, potential losses to the air phase, and the quantitative outcome of volatile organic compound analysis. Figures showing a retention index verification confidence assignment plot, boiling point versus 1D retention model confidence assignment plots, effect of retention index verification filter, and hopane biomarker patterns and representative ratios. (PDF)

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Notes

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Endocrine disrupting activities of surface water associated with a West Virginia oil and gas industry wastewater disposal site



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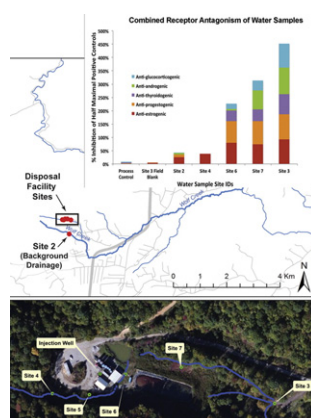
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HIGHLIGHTS

- Oil and gas wastewater disposal may increase endocrine disrupting activity in water.
- Tested EDC activity in surface water near oil and gas wastewater injection site.
- Water downstream had significantly more EDC activity than reference water upstream.
- Downstream surface water antagonized five different nuclear hormone receptors.
- EDC activity downstream was above levels known to result in adverse health effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Currently, >95% of end disposal of hydraulic fracturing wastewater from unconventional oil and gas operations in the US occurs via injection wells. Key data gaps exist in understanding the potential impact of underground injection on surface water quality and environmental health. The goal of this study was to assess endocrine disrupting activity in surface water at a West Virginia injection well disposal site. Water samples were collected from a background site in the area and upstream, on, and downstream of the disposal facility. Samples were solid-phase extracted, and extracts assessed for agonist and antagonist hormonal activities for five hormone receptors in mammalian and yeast reporter gene assays. Compared to reference water extracts upstream and distal to the disposal well, samples collected adjacent and downstream exhibited considerably higher antagonist activity for the estrogen, androgen, progesterone, glucocorticoid and thyroid hormone receptors. In contrast, low levels of agonist activity were measured in upstream/distal sites, and were inhibited or absent at downstream sites with significant antagonism. Concurrent analyses by partner laboratories (published separately) describe the

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Injection well
Wastewater disposal

analytical and geochemical profiling of the water; elevated conductivity as well as high sodium, chloride, strontium, and barium concentrations indicate impacts due to handling of unconventional oil and gas wastewater. Notably, antagonist activities in downstream samples were at equivalent authentic standard concentrations known to disrupt reproduction and/or development in aquatic animals. Given the widespread use of injection wells for end-disposal of hydraulic fracturing wastewater, these data raise concerns for human and animal health nearby.

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1. Introduction

It has recently been demonstrated that chemicals used in and/or produced by unconventional oil and natural gas (UOG) operations include endocrine disrupting chemicals (EDCs) (Bolden et al., 2015; Kassotis et al., 2014; Webb et al., 2014). EDCs are exogenous chemicals or mixtures of chemicals that can interfere with any aspect of hormone action (Zoeller et al., 2012). As many as one thousand EDCs have been identified (TEDX, 2013), both synthetic and naturally occurring, that can directly interact with hormone receptors as agonists or antagonists (Tyler et al., 1998; Yang et al., 2006), or indirectly interact via modulating responses to endogenous hormones (Chen et al., 2007; Jansen et al., 2004), endogenous hormone levels (Chen et al., 2007; Hayes, 2002), or through other mechanisms (Diamanti-Kandarakis et al., 2009). EDCs can exhibit biological effects at very low environmental concentrations (Roepke et al., 2005), can exhibit non-monotonic response curves (quantitatively and qualitatively different outcomes at low versus high concentrations), and can alter development during critical windows and increase the risk of disease (Vandenberg et al., 2012; Welshons et al., 2003).

UOG extraction involves harvesting oil and natural gas reserves, including shale gas, coal bed methane, and shale oil, trapped in impermeable or low-permeability geologic layers. As such, extraction of these energy resources requires stimulation, routinely via processes such as hydraulic fracturing (high pressure injection of water, chemicals, and suspended solids), to fracture the target layer and release the trapped natural gas and/or oil (Waxman et al., 2011; Wiseman, 2008). While less than fifty chemicals are typically used for the hydraulic fracturing of a single well, there are approximately 1000 different chemicals used by industry across the US (US EPA, 2015; Waxman et al., 2011); of these, >100 are known or suspected EDCs (Colborn et al., 2011; Kassotis et al., 2014; Waxman et al., 2011). A small percentage of injected fluids are recovered as “flow back” over approximately the first two weeks, while “produced water” is then generated over the life of the producing well (Deutch et al., 2011; Engle et al., 2014). These wastewaters can be heavily laden with naturally occurring radioactive compounds, heavy metals, and other compounds from the shale layer (Akob et al., 2015; Rowan et al., 2015), as well as chemicals and compounds used and produced by fracturing operations, and are routinely injected into disposal wells, reused in future fracturing operations, and/or pumped into open evaporation pits for disposal (Deutch et al., 2011; Lee et al., 2011; Lester et al., 2015; Wiseman, 2008).

Economically feasible methods to treat and reuse hydraulic fracturing wastewater are still under development, so injection remains the major disposal method, despite concerns over associations between injection disposal wells and increased seismicity and earthquakes (Ellsworth et al., 2015; Weingarten et al., 2015). More than 95% of produced wastewater in the US is injected for final disposal (US EPA, 2015; Clark and Veil, 2009), though centralized wastewater disposal facilities handle a more significant portion of wastewater in the Marcellus Shale region specifically (US EPA, 2015; Lutz et al., 2013). Spills and/or discharges of wastewater have been shown to increase: 1) fracturing chemical concentrations in local water supplies and sediments (DiGiulio et al., 2011; Rozell and Reaven, 2012; Skalak et al., 2014), 2) heavy metals in drinking water (Fontenot

et al., 2013; Jackson et al., 2013; Osborn et al., 2011), and 3) radioactivity, salinity, and total dissolved solids in rivers downstream from treatment plants and/or discharges (Harkness et al., 2015; Hladik et al., 2014; Warner et al., 2013), potentially leading to the production of disinfection byproducts (Harkness et al., 2015; Hladik et al., 2014; Parker et al., 2014). Previous work in our laboratory has reported potential human and animal health concerns via UOG contamination (Kassotis et al., 2014, 2015c; Webb et al., 2014) as well as adverse health outcomes in male C57 mice exposed during gestation to potentially environmentally-relevant concentrations of a hydraulic fracturing chemical mixture (Kassotis et al., 2015b). Because of these health concerns and the many potential contamination pathways (spills during transport to/from sites, improper handling and disposal of wastewater, failure of well casings, etc.), it is important to fill key data gaps in understanding contamination via underground injection activities and potential environmental impacts (US EPA, 2015).

As such, the goals of this study were to characterize the endocrine disrupting activities of water samples collected from a site where the chemical analyses indicated release of UOG wastewater had occurred and to ascertain potential health risks. Due to the high degree of conservation in nuclear receptor pathways (Diamanti-Kandarakis et al., 2009), in vitro screens such as reporter gene assays and yeast receptor screens are commonly used to assess potential health effects in human and wildlife populations (Naylor, 1999; Soto et al., 2006). These in vitro screens can more easily assess potential threats to human and environmental health than more costly and time-consuming animal studies, since the ability of a chemical to interfere with any aspect of hormone action is a clear indicator of potential resultant health outcomes (Zoeller et al., 2012). Mammalian reporter gene assays are often used due to high sensitivity and the translational potential of results (Naylor, 1999; Soto et al., 2006). Yeast receptor screens tend to be less sensitive, though are less susceptible to toxicity (Leusch et al., 2010). Due to these factors, we opted to couple mammalian and yeast bioassays to assess differences between the systems and to ensure that toxicity concerns would not prevent characterization of EDC activities at these sites. We further used authentic standards to convert receptor activities to equivalent concentrations of well-described control chemicals, facilitating the translation of in vitro results, as exposure to EDCs has been linked to a number of negative health outcomes in laboratory animals at environmentally relevant concentrations, wildlife and humans (Akingbemi and Hardy, 2001; Christiansen et al., 2008; Kelce and Wilson, 1997; Kidd et al., 2007; Mendiola et al., 2011; Sumpter and Jobling, 1995; Tyler et al., 1998).

The site examined herein was a West Virginia wastewater injection disposal facility that included an injection disposal well, several lined holding ponds and brine storage tanks, and a small stream that flows through the site (Fig. 1). This stream flows into the Wolf Creek downstream, and eventually into the New River, a drinking water source for local communities and important recreational area. A second tributary of Wolf Creek was identified as a background, non-impacted site, and samples were collected from both streams and assessed for agonist and antagonist activities for the estrogen (ER), androgen (AR), progesterone (PR), glucocorticoid (GR), and thyroid (TR) receptors. From our prior work with individual UOG chemicals and mixtures, we hypothesized that the disposal facility

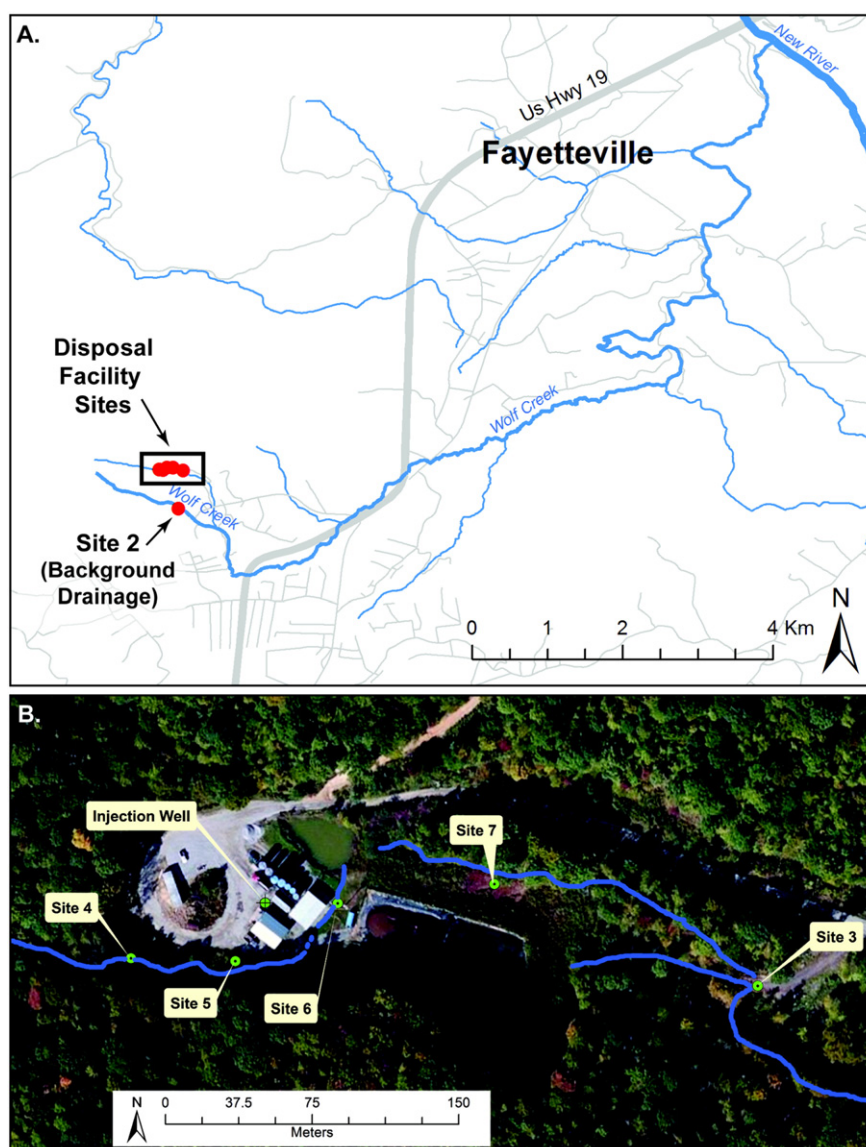


Fig. 1. Map of sampling locations. Map of sampling locations near Fayetteville, WV within the Wolf Creek watershed (A) and specific sites (B) in a stream running adjacent to a class II disposal facility. Panel A shows that Site 2 was located in a separate drainage from the disposal facility sites (outlined in black box), which are shown in panel B (Sites 4, 5, 6, 7 and 3). In panel B, the blue line highlights the stream as it flows through the disposal site. Water samples were not collected at Sites 1 and 5 for the work described herein.

Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographies, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community.

may contribute antagonist activities to the stream that could impact local health.

2. Materials and methods

2.1. Chemicals

17 β -Estradiol (E2; estrogen agonist, 98% pure), ICI 182,780 (estrogen antagonist, 98% pure), 4,5 α -dihydrotestosterone (DHT; androgen agonist, $\geq 97.5\%$ pure), flutamide (androgen antagonist, 100% pure), 3,3',5-triiodo-L-thyronine (T3; thyroid agonist, $\geq 95\%$ pure), progesterone (P4; progesterone agonist, $\geq 99\%$ pure), mifepristone (glucocorticoid/progesterone antagonists, $\geq 98\%$ pure), dexamethasone (DXM; glucocorticoid agonist, 99.5% pure), and hydrocortisone (glucocorticoid agonist, 98% purity) were purchased from Sigma-Aldrich Co. (St. Louis, MO). 1-850 (thyroid antagonist, $\geq 95\%$ pure) was purchased from EMD Millipore (Billerica, MA). Stock solutions were prepared at 10 mM in HPLC-grade methanol and stored at $-20\text{ }^{\circ}\text{C}$, (except T3 and 1-850, which were prepared

in dimethylsulfoxide; DMSO), and then diluted in respective solvents to required working solution concentrations.

2.2. Selection of sample sites and controls

Water samples ($n = 6$) were collected from surface water sites in June 2014 (Fig. 1, SI 2), including four sites associated with the disposal well: one sample collected upstream from the injection well (Background, Site 4), one near the injection well (Site 6), and another two samples downstream (Sites 7 and 3). Samples were collected from an additional background site in a separate drainage ("background drainage") with no known oil and gas wastewater inputs (Site 2). For additional information about the sampling sites, see Akob et al. (2016, unpublished results).

Process controls were prepared using one liter of Fisher HPLC-grade water (Fisher Scientific catalog # WFSK-4) and followed the same processing and analysis procedures used for all experimental samples. Process controls were included in assays to assess any receptor activities contributed by the solid phase extraction process.

2.3. Grab sample collection

All samples for Lab One (mammalian assays) were collected in one-liter amber glass bottles (Thermo Scientific catalog # 05-719-91) and samples for Lab Two (yeast assays) were collected in one-liter amber glass bottles (C&G Containers and Scientific Supplies, Lafayette, LA), all certified to meet the EPA standards for metals, pesticides, volatiles, and non-volatiles. Surface water samples were taken from flowing stream water by submerging bottles, filling completely, and capping without headspace. Samples for mammalian assays were preserved in the field by adding 1 g of sodium azide. A duplicate sample was collected at Site 3 and processed separately as an internal control (Supplemental information 2). Field blanks were collected at Site 3, and contained one liter of laboratory control water, opened and briefly exposed to the air, and then preserved and processed in the same manner as field samples. All samples were stored on ice in the field, shipped in coolers overnight to analysis labs, corrected to pH 3 with 6 N HCl (yeast assays only), stored at 4 °C in the respective laboratories, and were processed within two weeks of collection. All analyses were performed blinded to sample identification using non-identifiable coded IDs, and chain of custody procedures followed throughout the shipping and receiving processes.

2.4. Extraction of water samples

The two laboratories followed similar but distinct solid-phase extraction (SPE) protocols, both utilizing Oasis HLB glass cartridges (Waters # 186000683) after a pre-filtration step using glass-fiber filters. Cartridges for mammalian assays were conditioned with 100% HPLC-grade methanol and 100% HPLC-grade H₂O. Water samples (1 L) were loaded onto the cartridge and washed with 5 mL of 5% methanol. Cartridges were then removed from the vacuum manifold and elution was performed with three 1-mL additions of 100% methanol into amber glass vials. A DMSO “keeper” at 50 µL was added to each vial before dry-down under a gentle stream of nitrogen gas and subsequent reconstitution in 200 µL of pure methanol, creating stock concentrations of 4,000× the original water concentration (80% methanol, 20% DMSO). Solid-phase extractions for yeast assay samples were performed as described previously (Ciparis et al., 2012). Briefly, cartridges were conditioned with 100% ethyl acetate, 50:50 methanol: dichloromethane, 100% methanol, and then pH-3 HPLC-grade deionized water. Water samples (800 mL) were loaded onto the cartridge and column dried for at least 30 min following loading. Elution was performed with 6 mL methanol into one glass tube and 6 mL 50:50 methanol:dichloromethane into a second. Samples were dried under nitrogen gas and pooled, and subsequent reconstitution in 1 mL of pure methanol created stock concentrations of 800× the original water concentration.

Reconstituted samples were stored at –20 °C, protected from light, until tested. In order to be applied to cells, stock samples were diluted 100 and/or 1000-fold in tissue culture medium, creating final concentrations, in contact with mammalian and yeast cells, of 40×/4× the original water concentration for mammalian assays and 8× for yeast assays. Select SPE extracts from yeast assays were also tested in mammalian assays and exhibited equivalent activities to mammalian extracts.

2.5. Mammalian hormone receptor activity assays

Ishikawa cells (Sigma cat # 99040201) were maintained and transiently transfected with plasmids as described previously (Kassotis et al., 2015b; Kassotis et al., 2014) for ER alpha, AR, PR B, GR, and TR beta. Cells were induced with dilution series of the positive/negative controls (SI 3) or of the water sample extracts, diluted in medium using a 1% methanol vehicle. Each treatment concentration for each

sample was performed in quadruplicate within each assay and each assay was repeated three times.

Receptor activities were compared to 1% methanol or 0.1% DMSO vehicle controls as necessary, depending on vehicle used. Chemical response was set as a fold induction relative to this vehicle control, prior to calculating relative responses to control agonists and/or antagonists. Agonist activities were then calculated as a percent activity relative to the maximal positive control responses of 200 pM E2, 3 nM DHT, 100 pM P4, 100 nM T3, and 100 nM DEX, for ER, AR, PR, TR, and GR receptor assays, respectively. Antagonist activities were calculated as a percent suppression or enhancement of the positive controls at their EC₅₀s (concentration required to exhibit half of maximal activity): 20 pM E2, 300 pM DHT, 30 pM P4, 2 nM T3, and 5 nM DEX, respectively. Equivalence values were then determined for each sample with significant activity (based on paired *t*-test) using these percent activities relative to positive control agonist and antagonist dose response curves (Supplemental Fig. 1). Non-significant percent activities, while reported in Fig. 2, did not have equivalent concentrations calculated for Figs. 3 and 4.

2.6. Sample toxicity

The two laboratories followed distinct toxicity test protocols. Mammalian assays assessed toxicity as follows: CMV-β-Gal activity was used in ER assays as a marker of cell number, and also used as a surrogate marker for sample toxicity as described previously (Kassotis et al., 2014). Any sample found to have deviated >15% from the activity of the vehicle and that exhibited a significant difference (based on paired *t*-test) was deemed toxic and excluded from antagonist analysis. As antagonist assays measure the reduction in luciferase expression, toxicity cannot be unpaired from antagonist action. As such, any sample found to exhibit toxicity at the 40× concentration (Sites 3 and 7) were excluded and only tested for antagonism at 4× where no significant toxicity was observed for any sample. Yeast assays assessed toxicity at 8× water concentration using yeast strain BLYR (Sanseverino et al., 2009). Strain BLYR was grown to an OD₆₀₀ of 0.5 and was then added to samples and incubated for 4 h at 30 °C. Toxicity was expressed as the percent reduction in bioluminescence relative to vehicle control (2.5% methanol). Samples were considered toxic if a 10% reduction (or greater) in bioluminescence was observed. No toxicity was observed for any samples in the yeast system.

2.7. Yeast bioreporter assays

The bioluminescent yeast estrogen screen (BLYES) was used to quantitatively assess ER alpha activity relative to 17β-estradiol. Strain BLYES was purchased from 490 BioTech. Yeast strains DSY-1555 and MCY-105 were used to assess AR and GR, respectively. These yeast reporter strains were obtained from Marc Cox (University of Texas at El Paso). Detection limits for these yeast strains in the culture conditions described below are BLYES, 0.31 ng/L of 17β-estradiol; DSY-1555, 0.80 ng/L of dihydroxytestosterone; DSY-105, 0.05 ng/L of hydrocortisone.

The BLYES assay was performed as described previously (Balsiger et al., 2010; Ciparis et al., 2012) with some modifications. Strains DSY-1555 and MCY-105 were grown in synthetic complete media lacking lysine, uracil and tryptophan (SC-LUW) or uracil, tryptophan and histidine (SC-UWH), respectively. Yeast was grown at 30 °C in a rotary incubator for 48 h. Yeast was diluted to an OD₆₀₀ of 0.25 and 95 µL was added to wells of solid bottom white microplates (Costar). Standards (1.5 × 10⁴–8 ng/well) and samples (5 µL) were then added and plates were incubated at 30 °C for 4 h. After this incubation, 100 µL of Tropix GalScreen in Buffer B (Applied Biosystems, Foster City, CA) was added to all wells and the plate incubated for an additional 2 h at 28 °C. The hormone induced chemiluminescent signal

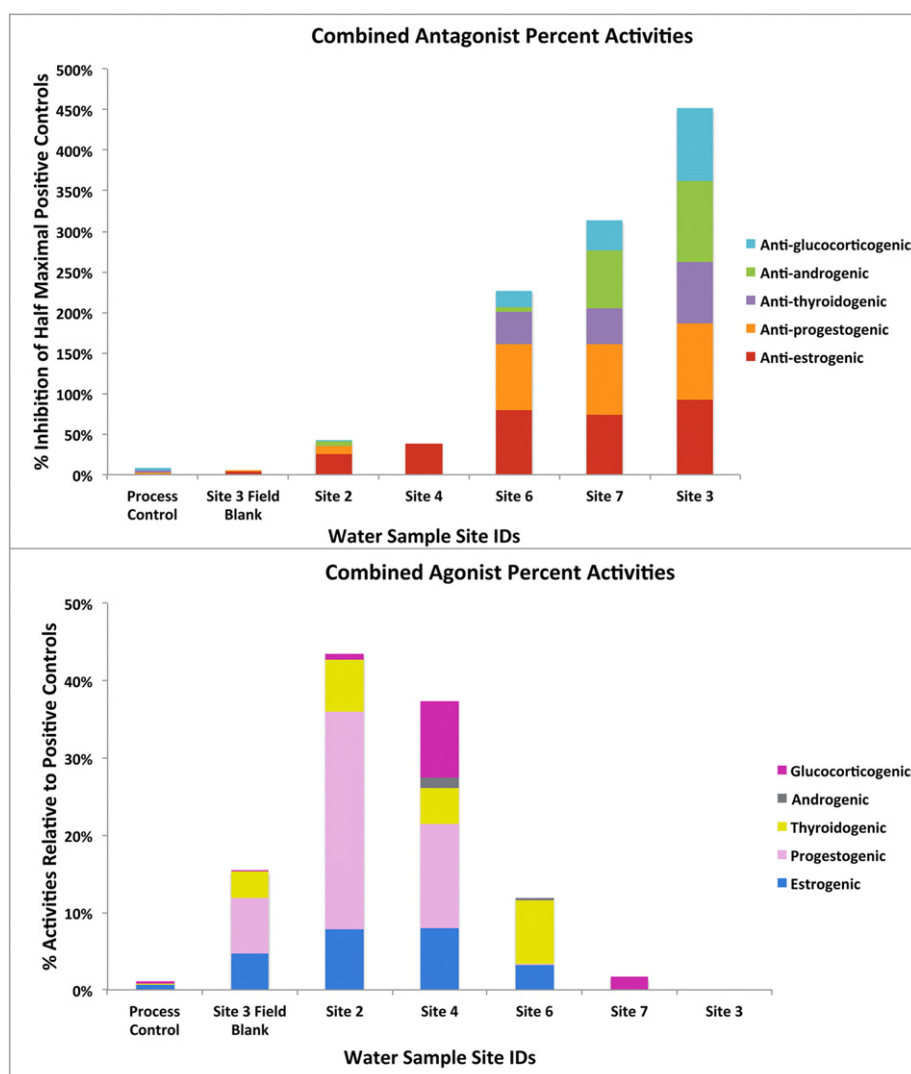


Fig. 2. Agonist and antagonist combined receptor activities of 4× surface water samples associated with injection well site via mammalian reporter gene assay. Combined total receptor activities for each water sample at 4× concentration. Combined total antagonist activities (A) as percent suppression of half maximal positive control response for each receptor. Combined total agonist receptor activities (B) as percent activity relative to maximal positive control response for each receptor. Results from duplicate samples, collected at Sites 7 and 3, were averaged and presented as one value for these sites. Samples are in order of degree of potential impact from the disposal facility: Process control, Site 3 field blank, Site 2 background stream, Site 4 upstream background, Site 6 adjacent to the injection well, Site 7 near former impoundment ponds, and Site 3 downstream of facility.

was then measured on a SpectraMax M4 microplate reader (Molecular Devices) in luminescence mode (1000 ms integration time).

3. Results

3.1. Antagonist receptor activities of water extracts

Increasing and near maximal antagonist activities were noted on and downstream of the disposal facility (Fig. 2). Site 6 (adjacent to the injection well) exhibited near maximal (>80%) antagonism for ER and PR, with antagonism for AR, GR, and TR increasing in Site 7 (adjacent to the impoundment ponds) and further in Site 3 (downstream of site; Fig. 2A). The background samples from the reference stream (Site 2) and from upstream of the disposal facility (Site 4) exhibited non-significant antagonism. Equivalence values were calculated based on positive control antagonists. Anti-AR and anti-TR equivalent activities were the highest measured, with levels reaching 700 µg EQ/L for each (flutamide and 1–850 equivalents, respectively; Fig. 3B, E). Anti-PR activity reached 5.5 µg mifepristone-EQ/L at Site 3 (Fig. 3C), anti-GR reached approximately 600 ng mifepristone-EQ/L (Fig. 3D), and anti-ER reached approximately 200 ng ICI-EQ/L (Fig. 3A).

3.2. Agonist receptor activities of water extracts

Low levels of background agonist activities (approximately 10-times lower than antagonist activities) were observed in the reference stream, Site 2, and the sample collected upstream from the wastewater disposal facility, Site 4 (Figs. 2B, 4). The main agonist activity was for PR, at 28% and 13% activity relative to the positive control at Sites 2 and 4, respectively (Fig. 2B). The field blank collected at Site 3 exhibited some non-significant agonist activities, and agonist activities were largely or completely gone in the three samples collected on or downstream from the injection disposal facility (Sites 6, 7, 3). Equivalence values at Sites 2, 4, and occasionally the field blank and background Site 6 exhibited low levels of agonist activities (Fig. 4). TR equivalence was highest, with levels as high as 5 ng T3-equivalents per liter water (T3-EQ/L; Fig. 4E), while both AR and GR equivalences were approximately 1 ng EQ/L (Fig. 4B, D). PR equivalence was between 115 and 150 pg P4-EQ/L (Fig. 4C), while ER was the lowest detected activity at 15–18 pg E2-EQ/L (Fig. 4A). All sites potentially impacted by injection fluids from the disposal well operations (Sites 6, 7, and 3) exhibited less agonist activities and considerably more antagonist activities than background samples.

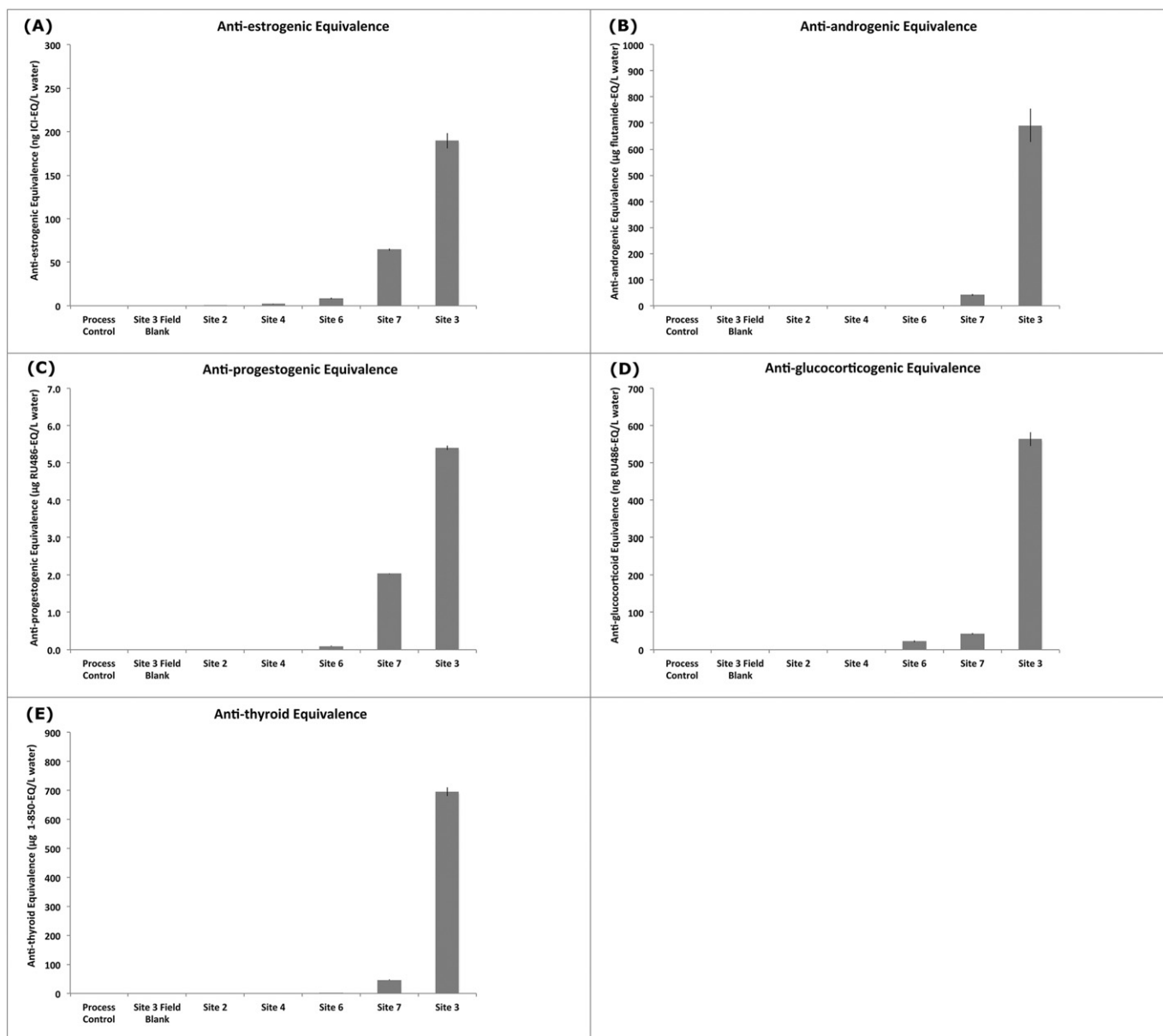


Fig. 3. Antagonist equivalence values of surface water samples associated with injection well site via mammalian reporter gene assay. Antagonist equivalences $\pm$ SEM calculated as an equivalent positive control antagonist concentration for: (A) anti-estrogenic (ICI), (B) anti-androgenic (flutamide), (C) anti-progestogenic (mifepristone), (D) anti-glucocorticoid (mifepristone), and (E) anti-thyroid (1–850) at each sample site at $4\times$ concentration. Results from duplicate samples, collected at Sites 7 and 3, were averaged and presented as one value for these sites. Equivalence values calculated only for samples exhibiting significant activity as described in the methods. Samples in order of increasing potential impact from facility.

ER, AR, and GR activities were also assessed in yeast reporter gene assays (SI 1). No significant GR activity was measured at any site. ER activity was only observed at the downstream Sites 7 and 3, with equivalent activities of approximately 350 and 650 pg E2-EQ/L (SI 1A). AR activity was likewise only measured at Sites 7 and 3, with equivalent concentrations between 1.3 and 1.5 ng DHT-EQ/L (SI 1B). No ER or AR agonist activities were observed at these sites in the mammalian reporter gene assays, though they exhibited the highest antagonism (Fig. 3).

3.3. Toxicity assessment of water extracts

Samples were assessed for toxicity via CMV- β -Gal activity in the ER activity screen in Ishikawa human cells, as described and validated previously (Kassotis et al., 2014). Briefly, a constitutively active promoter, CMV- β -Gal, was transfected into all cells alongside the reporter and receptor constructs for each experimental system. Any cell thus transfected with this promoter produced beta galactosidase, and

this could be measured as a marker for cell number and thus also as a surrogate marker for cell toxicity (Kassotis et al., 2014). Sites 7 and 3 both exhibited moderately high toxicity ($>60\%$ inhibition of beta-galactosidase production) at the $40\times$ test concentration (Fig. 5). None of the $4\times$ concentrations of these samples exhibited significant toxicity. Agonist and antagonist activities were only reported for samples without significant toxicity, so $4\times$ water concentration values were used to report all activities. No toxicity was observed for any samples at $8\times$ water concentration in the yeast reporter gene assay.

4. Discussion

We measured significantly greater EDC activity on and downstream of the disposal facility (Sites 6, 7, and 3) relative to reference sites (Sites 2 and 4). The impacted sites exhibited considerably greater antagonist activities than background samples (Figs. 2, 3, 4). These samples included Site 6 collected directly adjacent to the injection well, Site 7 collected

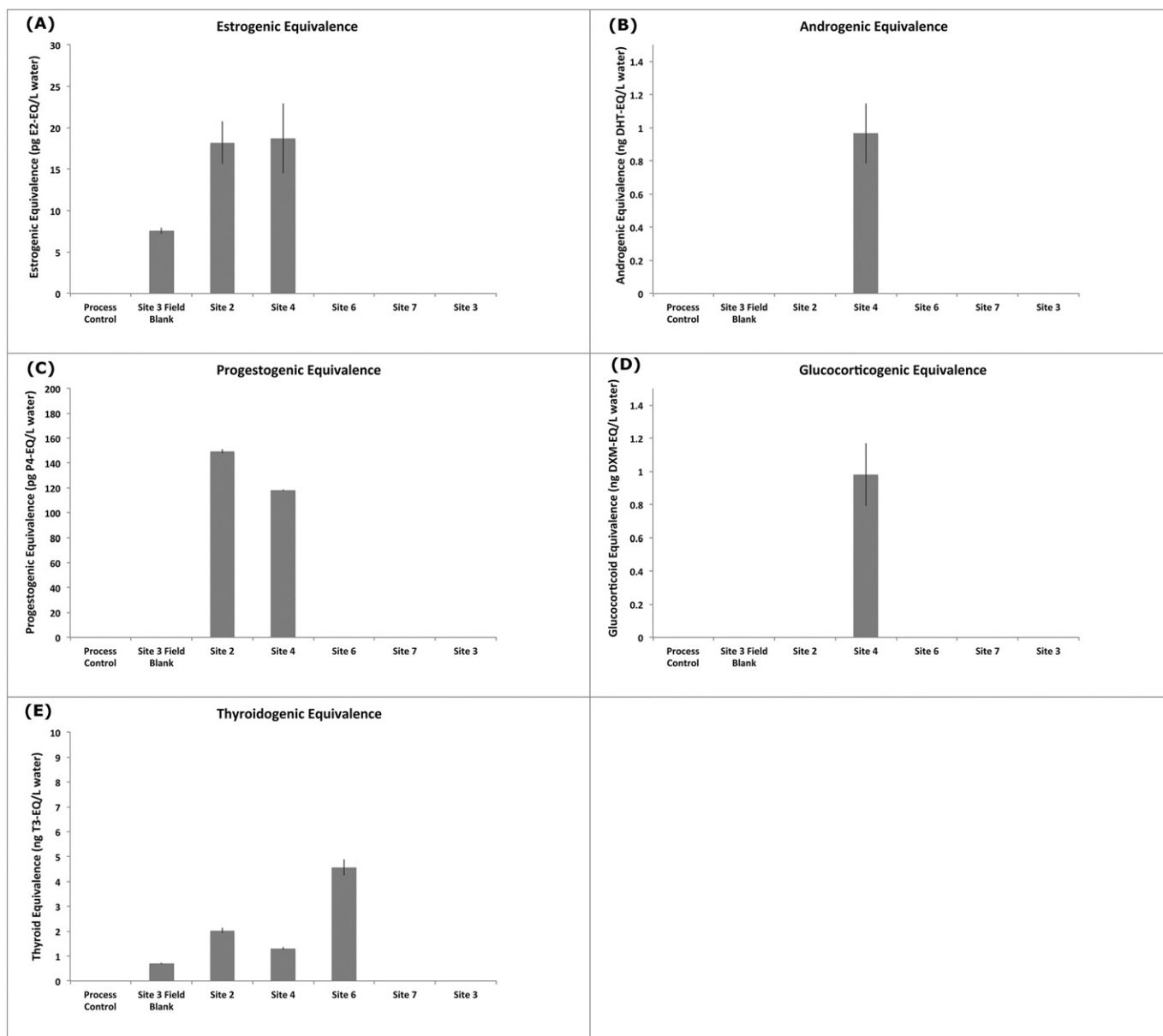


Fig. 4. Agonist equivalence values and receptor activities of surface water samples associated with injection well site via mammalian reporter gene assay. Agonist equivalences $\pm$ SEM calculated as an equivalent positive control agonist concentration for: (A) estrogenic (E2), (B) androgenic (DHT), (C) progestogenic (progesterone), (D) glucocorticoid (dexamethasone), and (E) thyroidogenic (T3) at each sample collection site at $4\times$ concentration. Results from duplicate samples, collected at Sites 7 and 3, were averaged and presented as one value for these sites. Equivalence values calculated only for samples exhibiting significant activity as described in the methods. Samples in order of increasing potential impact from facility.

immediately next to the former wastewater containment ponds, and Site 3 collected downstream from the entire disposal facility. Site 3 exhibited the most antagonism, likely due to this sample receiving drainage from the entire facility. The most impacted samples, Sites 7 and 3, also exhibited toxicity in the mammalian cell culture system at the $40\times$ concentration, but not at the $4\times$ concentration used to measure EDC bioactivity, nor at $8\times$ in the yeast cell culture system.

As a class II injection well, this site is permitted to accept wastewater from unconventional oil and natural gas extraction. However, this site may accept wastewater and fluids from other industries as well, and the hormonal activity profile exhibited may be due in part to other sources. As such, caution should be taken in the extrapolation of these results to unconventional oil and gas activities specifically. To address this concern, research performed concurrently (Akob et al., 2016 and Orem et al., 2016, unpublished results) describes in detail the analytical and geochemical profiling that identified inorganic and organic constituents indicative of UOG wastewater at these sites. Specifically, elevated

conductivity, sodium, chloride, and barium concentrations, and strontium isotopes suggest that the contamination profile is specifically due to the handling of UOG wastewater from shale gas and coal bed methane production (SI 2, Akob et al., 2016, unpublished results). In addition, numerous organic chemicals were identified in water and sediments downstream of the injection facility, many associated with UOG operations (Orem et al. 2016, unpublished results).

Several known hormonally active compounds were detected in the water near the injection disposal facility. However, differences in sensitivity and efficacy between assay systems prevent clear associations with degree of effects. Detected at Sites 3 and 7, tris(1-chloro-2-propyl)phosphate has been reported to act as an antagonist for the androgen (Weiss et al., 2011) and thyroid (Farhat et al., 2013) receptors. Detected at Site 3, 2-(2-butoxyethoxy)-ethanol (diethylene glycol methyl ether) has been tested by our lab previously and exhibited antagonistic activities for ER, AR, and GR (Kassotis et al., 2015b), though only at concentrations approximately 100-times above the $0.54\text{ }\mu\text{g/L}$

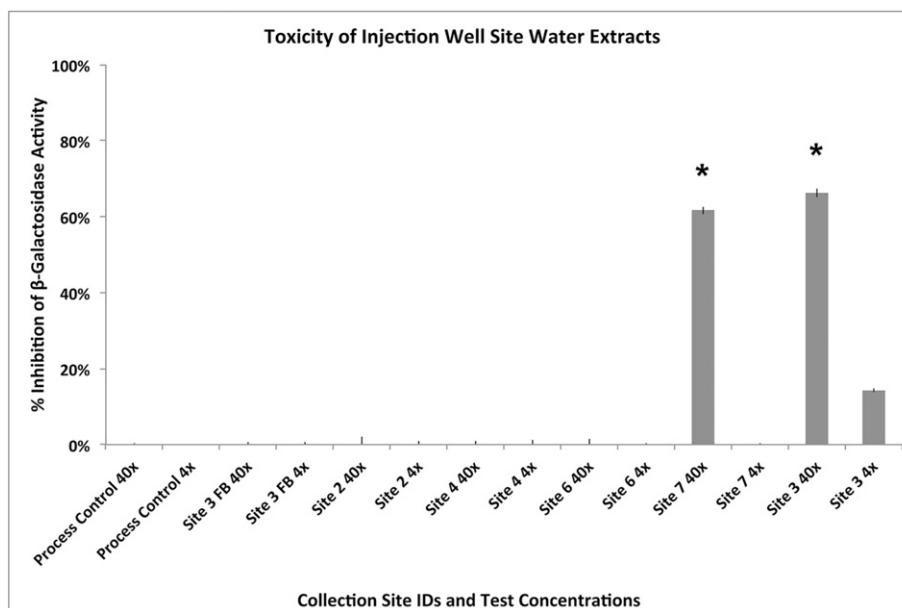


Fig. 5. In vitro toxicity of injection well site surface water samples. Toxicity as per inhibition of constitutively active promoter, CMV- β -Gal, measured as percent inhibition of β -galactosidase by 40 $\times$ and 4 $\times$ concentrations of each water sample $\pm$ SEM in Ishikawa cells. Significant inhibition designated by $>15\%$ inhibition of signal and significantly lower response than vehicle control, as per *t*-test. Results from duplicate samples, collected at Sites 7 and 3, were averaged and presented as one value for these sites. * $p < 0.05$ and $>15\%$ inhibition of vehicle control.

in the water at this site. Notably, many EDCs are hydrophobic and partition more readily into sediments, resulting in lower concentrations in water samples (Lai et al., 2000; Langston et al., 2005; Petrovic et al., 2001; Pojana et al., 2007). As such, sediments at Sites 3 and 7 contained 16 and 65 of the chemicals assessed, respectively. Future work should fractionate water samples to gain a clearer understanding of the chemicals driving the majority of antagonist activities.

The bioactivities we measured in water sample extracts from impacted sites were within the range known to impact the health of aquatic organisms. Specifically, anti-PR, anti-GR, anti-AR, anti-TR, and anti-ER activities were approximately 1000, 100, 30, 15, and 6 times greater than those known to disrupt the endocrine system in aquatic organisms, respectively (Bhatia et al., 2015; Bluthgen et al., 2013a, 2013b; Navarrete-Ramirez et al., 2014; Roepke et al., 2005). For example, 30 ng/L ICI inhibits development of sea urchins (Roepke et al., 2005), 25 μ g/L flutamide can induce vitellogenin production in fish (Bhatia et al., 2015), 5 ng/L mifepristone impacts egg production, disrupts folliculogenesis, and alters gene expression in zebrafish (Bluthgen et al., 2013a, 2013b), and 46 μ g/L 1–850 can alter gene expression in tilapia (Navarrete-Ramirez et al., 2014). Notably, antagonist equivalent activities reported downstream of the disposal facility (Sites 7 and 3) were above levels associated with adverse health effects in aquatic organisms for all five receptors. In many cases, even with considerable dilution, levels of endocrine disrupting contaminants would still be capable of disrupting the development of fish, amphibians, and other aquatic organisms.

Impacted sites largely contained minimal agonist activity, and generally occurred below levels known to impact aquatic wildlife. Agonist activities in water sample extracts from reference sites were also below those known to cause adverse health effects in aquatic organisms, to the best of our knowledge. Importantly, despite this low potential for disruption through single receptor mechanisms, adverse health effects may result from disruption of several receptor pathways simultaneously. For example, Runnalls et al. recently reported that ER, AR, and PR agonist pathways could all result in inhibition of egg production in fathead minnows through separate mechanisms (Runnalls et al., 2015), suggesting that some endpoints may require a more comprehensive approach than assessing equivalent concentrations for individual receptors.

Our lab has previously reported anti-ER and anti-AR equivalences in surface and groundwater collected from drilling-dense sites with a history of hydraulic fracturing fluid spills in Colorado (Kassotis et al., 2014) and surface water impacted by wastewater effluent in Missouri (Kassotis et al., 2015a), though equivalent ER and AR agonist and antagonist concentrations were much lower than those described herein. For example, wastewater effluent impacted streams in Missouri exhibited up to 19 ng/L ICI equivalence (anti-ER) and 48 μ g/L flutamide equivalence (anti-AR), approximately 10 and 14-fold lower activities than detected in this study. Other researchers have utilized similar in vitro screens to assess the EDC activities contributed by various anthropogenic sources to water, though varying positive controls and assay sensitivities complicate comparisons. However, wastewater is a well-described source of anti-androgens, with raw sewage containing up to mg/L levels of flutamide equivalence (Ma et al., 2013). Researchers assessing the Lambro River in Italy, heavily contaminated with domestic and industrial wastewater and agricultural run-off, reported 370–4700 μ g/L flutamide equivalences (Urbatzka et al., 2007). Similarly, assessment of the Pearl River System in China, heavily contaminated by effluent and raw sewage from four major wastewater treatment plants, exhibited 20–935 μ g/L flutamide equivalence and up to 1.3 mg/L tamoxifen equivalence (anti-ER; Zhao et al., 2011). While tamoxifen exhibits agonist activity in our uterine bioassay system and thus cannot be readily compared, the anti-androgenic activities were similar to ours.

Differences noted between the activities exhibited in the mammalian and yeast screens are likely due to several known factors. Many of the tissue-specific effects of EDCs in mammalian systems are due to the varied expression of coregulatory proteins recruited by the ligand-bound receptor complex (Diel, 2002; Shang and Brown, 2002). Yeast receptor screens lack many of these coregulators, and chemicals that act as antagonists in mammalian systems can act as agonists in a yeast system (Lyttle et al., 1992; Sohoni and Sumpter, 1998; Urbatzka et al., 2007). Yeast cells also lack some enzymes that are commonly expressed in mammalian cells, preventing bioactivation of some chemicals routinely observed in mammalian cells (Bovee et al., 2007). Lastly, the permeability of chemicals through the cell wall in yeast is different from mammalian cell membranes, resulting in differential sensitivity to various chemicals (Wilson et al., 2004).

5. Conclusions

In conclusion, we report high levels of EDC activities in surface water extracts associated with a wastewater injection disposal facility. The most impacted sites were on and downstream from the disposal facility (Sites 6, 7, and 3), and exhibited considerably more antagonist activities and less agonist activities than background samples (Figs. 2, 3, 4). The most impacted samples, Sites 7 and 3, also exhibited toxicity in the mammalian cell culture system at the $40\times$ concentration, but not at the $4\times$ concentration that we used to measure EDC activity. Importantly, the water leaving this site exhibited nuclear receptor equivalent activities that are known to result in adverse health effects in aquatic organisms and other animals. While Wolf Creek flows into the New River, a drinking water resource, this sampling occurred approximately 5 miles upstream from the confluence. Further work should assess how the magnitude of EDC effects changes with distance from the site in order to better assess potential human and animal health threats from exposure. Given the large number ($>140,000$) of class II injection wells currently operating in the United States, this should be viewed as a case study of environmental impacts that may be evident at other injection disposal facilities as well. Further work, including higher tier receptor disruption screens (whole cell activity as well as fish, amphibian, and mammalian whole animal assessments) should be applied to confirm these results and assess water quality surrounding these facilities in a more comprehensive manner.

Competing financial interest declaration

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.03.113>.

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Unconventional oil and gas spills: Materials, volumes, and risks to surface waters in four states of the U.S.

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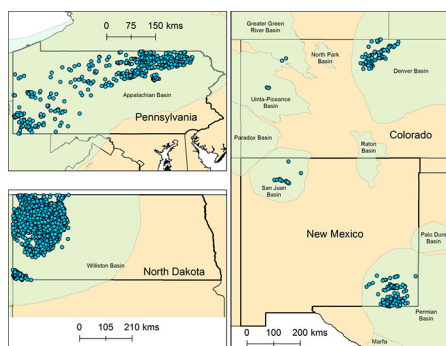
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HIGHLIGHTS

- Analyzed data from 6622 spills from horizontal UOG wells in four U.S. states
- Wastewater, crude oil, HF solution and drilling waste were most often spilled
- Average distance of spills to the nearest stream was smallest in Pennsylvania
- Some spills in all states occurred within current surface water setback regulations
- Pennsylvania spills occurred in watersheds of higher importance to drinking water

GRAPHICAL ABSTRACT



Distribution of spills attributed to unconventional oil and gas wells by state. Light green polygons indicate shale basins (basin nomenclature and shapefile from USEIA (2011)).

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ABSTRACT

Extraction of oil and gas from unconventional sources, such as shale, has dramatically increased over the past ten years, raising the potential for spills or releases of chemicals, waste materials, and oil and gas. We analyzed spill data associated with unconventional wells from Colorado, New Mexico, North Dakota and Pennsylvania from 2005 to 2014, where we defined unconventional wells as horizontally drilled into an unconventional formation. We identified materials spilled by state and for each material we summarized frequency, volumes and spill rates. We evaluated the environmental risk of spills by calculating distance to the nearest stream and compared these distances to existing setback regulations. Finally, we summarized relative importance to drinking water in watersheds where spills occurred. Across all four states, we identified 21,300 unconventional wells and 6622 reported spills. The number of horizontal well bores increased sharply beginning in the late 2000s; spill rates also

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Hydraulic fracturing
Extraction
Spill rates
Wells
Colorado
New Mexico
North Dakota
Pennsylvania
Setback regulations

increased for all states except PA where the rate initially increased, reached a maximum in 2009 and then decreased. Wastewater, crude oil, drilling waste, and hydraulic fracturing fluid were the materials most often spilled; spilled volumes of these materials largely ranged from 100 to 10,000 L. Across all states, the average distance of spills to a stream was highest in New Mexico (1379 m), followed by Colorado (747 m), North Dakota (598 m) and then Pennsylvania (268 m), and 7.0, 13.3, and 20.4% of spills occurred within existing surface water setback regulations of 30.5, 61.0, and 91.4 m, respectively. Pennsylvania spills occurred in watersheds with a higher relative importance to drinking water than the other three states. Results from this study can inform risk assessments by providing improved input parameters on volume and rates of materials spilled, and guide regulations and the management policy of spills.

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1. Introduction

Development of oil and gas from unconventional shale sources (unconventional oil and gas, or UOG) has dramatically increased over the past ten years in large part due to the combination of horizontal drilling and hydraulic fracturing. Horizontal drilling refers to the process where a wellbore aligns horizontally with the target formation, thus increasing contact with the reservoir, and hydraulic fracturing refers to the process that stimulates oil and gas within the reservoir by expanding fractures in shale through injection of fracturing fluid (i.e., water, proppant and chemicals) (USDOE, 2009). The U.S. is currently the leader in developing UOG resources, where from 2000 to 2016 daily production of shale gas (dry) increased by 20-fold (2.2 to 44.0 billion cubic feet) and tight oil increased by >10-fold (0.4 to >4.5 million barrels) (USEIA, 2016). Other countries are beginning to commercially produce oil and gas from shale and low-permeability formations (USEIA, 2015), and by 2040, unconventional gas production is projected to triple to account for almost a third of global natural gas production (IEA, 2015). Given the rapid, recent development of UOG, data are scarce on its long-term environmental impacts, and there is a need to better quantify risk to people and nature (Finkel and Hays, 2013; Small et al., 2014; Souther et al., 2014; Werner et al., 2015).

UOG development can affect species, ecosystems, and the services they provide to people. In central North America, estimates suggest that oil and gas development (including coal bed methane) reduced net primary productivity, an important measure of a region's ability to provide ecosystem services, by ~4.5 Tg of carbon from 2000 to 2012 (Allred et al., 2015). Further, land application of hydraulic fracturing fluid resulted in leaf drop and 56% mortality of trees where the application occurred (Adams, 2011). Forest interior bird counts increased with distance from a well pad in Pennsylvania (Barton et al., 2016), abundances of sagebrush songbirds decreased with increased well density in Wyoming (Gilbert and Chalfoun, 2011), and mule deer have been documented to avoid well pads with active drilling by at least 800 m in Colorado (Northrup et al., 2015). In Kentucky, an accidental release of hydraulic fracturing fluid into a stream increased gill lesions and other indicators of stress in fish (Papoulias and Velasco, 2013), and in Pennsylvania, juvenile mussels below a brine treatment plant had lower survival rates than mussels located above the plant (Patnode et al., 2015). Streambed microbial diversity was lower below an oil and gas waste injection plant in West Virginia (Akob et al., 2016), and water downstream from this site had higher endocrine-disrupting activities than reference water (Kassotis et al., 2016). Despite the emerging evidence, studies establishing causal relationships between environmental changes and UOG activities are scarce; this is particularly true for spills and releases of materials used in and produced by UOG development.

Summary reports on UOG spills are starting to emerge; however, they are typically restricted to a single state, short on detail regarding materials spilled or reasons for spills, or are characterized by a small sample size. For example, the Colorado Oil and Gas Conservation Commission (COGCC, 2014) reported that equipment failure and human error were the two main causes of spills, most spills occurred during

the production stage, process piping, pipelines and tanks were the main sources of spills, and the volume of 12% of the spills were >100 barrels (15,900 L); however no detailed analysis on spilled material was presented. Brantley et al. (2014), using the Pennsylvania Notice of Violation (NOV) database, reported that one-fifth of wells were given a non-administrative violation from 2005 to 2013, and Rahm et al. (2015) reported that Pennsylvania NOV (2007–2013) related to spills and erosion were the most common NOV issued. Neither study, however, conducted a detailed analysis on volumes or materials spilled or their potential impacts to surface waters in Pennsylvania. Finally, the U.S. Environmental Protection Agency (USEPA, 2015a) reviewed over 36,000 spill records from nine states but was able to confidently identify only 457 incidents associated with hydraulic fracturing (~12,000 contained insufficient information and ~24,000 were not related to hydraulic fracturing). The USEPA reported most spills were small (<1000 gal, 3785 L), flowback and produced waters were the most commonly spilled material, human error was the most common cause of a spill, storage units were the common source of spills, and 300 of the spills reached an environmental receptor; however, the study did not include spills that occurred during the drilling stage.

The objectives of this study were to characterize the volumes and compositions of the materials spilled from horizontal, hydraulically fractured oil and gas wells, and evaluate the risk that spills posed to streams and surrounding watersheds important to human drinking water. Our first objective aimed to fill the knowledge gap on the materials and volumes spilled during UOG development. Our second objective focuses on streams because they provide habitat that supports a high level of biodiversity (Meyer et al., 2007), are particularly vulnerable to UOG development due to their tight coupling with upstream catchments (Hynes, 1975), and are sensitive to small changes in catchment conditions from anthropogenic activities (Maloney et al., 2012). Further, over 1/3 of the U.S. population uses public drinking water systems that rely, at least in part, on intermittent, ephemeral or headwater streams (USEPA, 2009). The spatial position of anthropogenic activities within the catchment often affects these relationships (King et al., 2005), which is especially important for UOG because wells are frequently located in close proximity to streams (Entekhin et al., 2011). We therefore evaluated the risk of spills to streams by quantifying the spatial position of spills to the nearest stream and how these distances related to current setback regulations. Because a large population relies on surface water for domestic use, our second objective also explored risks to drinking water using the U.S. Forest Service's Forest to Faucets data set. We provide a broad analysis of spill features to improve understanding of the potential environmental risks of spilled materials from UOG development and to inform management practices and policy formulation.

2. Study site and methods

2.1. Study sites and setback regulations

We sampled state databases on spill records for four states (Colorado – CO, New Mexico – NM, North Dakota – ND, and Pennsylvania – PA)

that have accessible oil and gas spill data and that are underlain by a number of shale basins (USEIA, 2011). Each of the states experienced an increase in horizontal hydraulic fracturing over the past decade (Fig. S1); however, they vary in production type from ND being primarily an oil producer to PA being primarily a gas producer. These states provide a representative range in information on spills related to UOG, which should be applicable to many geographic and ecological settings. Each state's laws and reporting requirements also vary for when (volume threshold or potential impact to people or water) and how spills are reported (verbal or written and reporting requirements) (CDPHE, 2009, NM Admin Code R. 19.15.29, ND Admin. Code R. 43-02-03-30, 25 Pa. Code § 78.66). These differences drive the quantity and quality of spills data reported in each state; therefore, a higher spill rate in a state may just reflect a more robust reporting system.

There is also considerable variability in stream setback regulations for UOG across the states examined in this study. In CO, new oil and gas development must generally avoid “restricted surface occupancy areas”, which are defined to include areas within 300 ft (91.4 m) of the ordinary high water mark of any stream segment located within designated Cutthroat Trout habitat, or streams or lakes designated by the Colorado Parks and Wildlife as “Gold Medal” (2 Colo. Code Regs. 404-1:100, 404-1:1205). Operators proposing to drill a well on a previously undisturbed site must first obtain a location assessment from the Colorado Oil and Gas Conservation Commission and must indicate on the form provided for this assessment that the proposed site is within a restricted surface occupancy area (2 Colo. Code Regs. 404-1:303b(3)(P)). Further, no new well may be drilled within 300 ft (91.4 m) of a public water system without a variance and consultation with the CO Department of Public Health and the Environment (2 Colo. Code Regs. 404-1:317B(c), and a well may only be located between 301 and 2640 ft (91.7 and 804.7 m) from a public water system if additional security measures are taken and baseline water quality testing is conducted (2 Colo. Code Regs. 404-1:317B(d)(4), (e)(2)).

In NM, wellhead protection areas include 200 ft (61.0 m) of a private, domestic freshwater well or spring used by less than five households or within 1000 ft (304.8 m) of any other freshwater well or spring (NM Admin Code R. 19.15.2.7(W)(8)). Further, permanent waste pits (NM Admin. Code R. 19.15.17.10(A)(5)) and recycling containments (NM Admin. Code R. 19.15.34.11) are not to be located within 300 ft (91.4 m) from a continuously flowing watercourse or 200 ft (61.0 m) from any other significant watercourse or lakebed, sinkhole or playa lake. Operators may obtain a waiver from these requirements if the state determines that waters will be protected from the permanent waste pit. Permanent waste pits and recycling containments also may not be located within 500 ft (152.4 m) of a spring or freshwater well, 500 ft (152.4 m) of a wetland, or within the 100 year floodplain.

In ND, Admin. Code R. 43-02-03-19 states that “[w]ell sites and facilities shall not be located in or hazardedly near, bodies of water, nor shall they block natural drainage”. However, no setback distances are specified.

In PA, the edge of the disturbed area associated with an UOG well previously had to be set back 100 ft (30.5 m) or the vertical portion of the well must be 300 ft (91.4 m) from the edge of any “solid blue lined stream, spring or body of water as identified on the most current 7 1/2 minute topographic quadrangle map of the United States Geological Survey.” (58 Pa. Stat. § 3215(b)). However, this setback requirement was enjoined in *Robinson Township v. Commonwealth*, 83 A.3d 901, 1000 (Pa. 2013) and is not in effect. Pennsylvania's Act 13 also allowed the Department of Environmental Protection to require that hazardous chemicals and materials used in UOG be stored 750 ft (229 m) from blue lined streams (58 Pa. Stat. § 3215(d.1)). Finally, the Commonwealth restricts well site placement within floodplains (58 Pa. Stat. § 3215(f)).

2.2. Spill data and rates

We analyzed spill data from 01 January 2005 through 31 December 2014 for NM, ND, and PA, and from 01 January 2005 through 31

December 2013 for CO because of a significant change in CO spill reporting in 2014 (COGCC, 2014) a time period that encompassed the majority of horizontal UOG well development in these states (Fig. S1). Colorado spill data were obtained on 2 September 2014 from the state's online incident report Form 19/19A (<http://cogcc.state.co.us/data.html#/cogis>). Spill data within NM were accessed on 25 June 2015 from the New Mexico Oil Conservation Division website on spills (<https://wwwapps.emnrd.state.nm.us/ocd/ocdpermitting/Data/Incidents/Spills.aspx>). North Dakota spill data were collated on 26 August 2015 from the oilfield environmental incident reports on the North Dakota Department of Health – Environmental health website (<https://www.ndhealth.gov/EHS/Spills/>). Pennsylvania does not have a spill database; instead spills are reported in a Notice of Violation (NOV) database (http://www.depreportingservices.state.pa.us/ReportServer/Pages/ReportViewer.aspx?/Oil_Gas/OG_Compliance). We downloaded all PA NOV's from this site on 03 April 2015 and using the “violation codes” listed classified each violation as a “spills, potential spills” similar to Rahm et al. (2015, Table S1).

Each spill or NOV record was individually examined for duplication, errors, and a description of material and volume spilled. We grouped materials into ten main categories: chemicals, condensate, crude oil, diesel fuel, drilling waste, freshwater, hydraulic fracturing solution, natural gas, sediment, and wastewater. Materials not falling into these ten categories were grouped under “other”. For consistency across states, we had to group some of the reported material classifications into these more general categories. Drilling mud, cuttings, and drilling fluid were not consistently reported across states so these were grouped together as “Drilling waste”; similarly brine, flowback, and produced water were grouped as “Wastewater”. Hydraulic fracturing solution and frac fluid were reclassified as “HF Solution”, and chemicals used in the development process (e.g., HCl, antifreeze, surfactant, and glycol) were classified as “Chemicals”. For some records more than one material was reported; for these we treated each material as a separate spill. In ND, 20 records had no material spilled and were removed from analysis. North Dakota also had three records with a zero value for volume spilled; we classified these records as no volume reported.

We obtained well information from 01 January 1995 through 31 December 2014 from the IHS Enerdeq database (IHS, 2016), a private source for well information that synthesizes and independently quality assures data from state agencies and organizes it in a user-friendly searchable format. We used this single, private source for well information to minimize data inconsistencies among states. Because there was no clear designation of what constituted an UOG well in this database, we used geologic province name, play type, well status, and well bore orientation to identify UOG wells. UOG geologic provinces included the Denver Basin, Green River Basin and Piceance Basin in CO; Williston Basin for ND; Permian Basin and San Juan Basin for NM and Appalachian Basin for PA. We therefore considered wells as UOG if they 1) overlapped with the mentioned geological provinces, 2) had a play type classified as Shale Gas, Tight Gas, or Tight Oil, 3) had a well status of oil, gas, abandoned, pilot or suspended designation, and 4) had a horizontal hole direction.

We merged the entire spills dataset to the UOG well dataset using the American Petroleum Institute (API) number, a unique number for all oil and gas wells drilled in the U.S. This resulted in a dataset that contained only those spills associated with horizontally drilled UOG wells since 1995. We calculated spill rates by material for each state using the number of spills for a given year divided by the cumulative number of spudded wells since 1995. We used the cumulative number of wells because we wanted to examine the risk of spills throughout the life of a well. We multiplied the resulting rates by 100 thus report spill rates per 100 wells.

2.3. Potential risks to people and nature

Because streams support numerous freshwater taxa, including those for human consumption, and provide a source of drinking water for

humans we assessed the potential risks of spills to these ecosystems two ways. First, we compared distances of spills to the nearest stream among states by measuring proximity (linear geographic distance) of spills to a stream using the NHDplusV2 high resolution flowline dataset (McKay et al., 2012) and the Near Tool in ArcGIS 10.2.2 (ESRI, Redlands, California, USA). We assumed that spills occurring closer to streams can pose a higher risk than spills occurring further away. Geospatial information of spills was not available in the state spill reports; therefore we used the latitude and longitude of wells as the spatial location of the spill. We acknowledge that some spills associated with supporting infrastructure and equipment of the well, e.g., storage tanks and flowlines, may not be located at close proximity to the well head, however locational data for such structures were not available for this analysis. We therefore used the well head coordinates as a surrogate, potentially over- or under-estimating actual distance of spills to streams; however, we have no reason to believe there would be a systematic bias for over- or under-estimation of distances. Second, using the distance to stream data, we calculated the number of spills in each state that occurred within the various state setback distances from streams listed above (30.5 m, 61.0 m, 91.4 m, 152.4 m and 228.6 m).

However, the importance of streams as sources of drinking water varies greatly across the U.S. Therefore, we also explored risks to drinking water among states using the U.S. Forest Service's Forest to Faucets data set (Weidner and Todd, 2011). Based on water production and water use, the Forest to Faucets data produced a Hydrologic Unit Codes (HUC) 12 watershed level index of relative importance to surface drinking water, which ranks relative importance from least important, 0, to most important, 100. We used this importance index to examine potential risk of UOG spills to surface drinking waters. All data analyses and figures were performed in R (R Development Core Team, 2015) or ArcGIS 10.2.2.

3. Results

3.1. Well and spill temporal patterns

We identified 6622 UOG spills from 5958 unique reports in the four states' databases (Table 1). Six hundred and fifty eight reports (11.1% of total) had more than one material reported on the same incident report. North Dakota had the most horizontal UOG wells, followed by PA, NM, and then CO (Table 1). While all states showed a sharp increase in the number of horizontal wells over time (Fig. S1), the number of spills increased sharply in the late-2000s only for ND and PA; for PA, the increase was followed by a decreasing trend (Fig. 1A). The maximum number of reported spills in one year was 78 in 2013 for CO, 170 in 2014 for NM, 1374 in 2014 for ND, and 324 spills in 2010 for PA (Fig. 1A, Table S2). Spill rate increased for all states except PA where the rate initially increased, reached a maximum of 20.3 spills per 100 wells in 2009 and then decreased (Fig. 1B, Table S2).

3.2. Spilled materials and volumes

We were able to identify materials for 6082 spills (91.8% of all spills). The predominant material spilled varied across states, with wastewater

Table 1

Number of wells and spills by state. "with volumes" column indicates the number of spills with volume data; "with materials" column shows the number of spills with materials spilled data. Numbers in parentheses indicate percentage of the number of wells (unique records, total) or percentage of total spills (with volumes, with materials). The unique records column indicates the number of unique spill reports; the Total column does not show total unique spills because we considered spill reports with more than one material as separate spills.

State	Number of wells	Number of spills			
		Total	Unique spill records	With volumes	With materials
Colorado	1753	139 (7.9)	125 (7.1)	135 (97.1)	136 (97.8)
New Mexico	2197	316 (14.4)	265 (12.1)	276 (87.3)	312 (98.7)
North Dakota	10,881	4986 (45.8)	4428 (40.7)	4859 (97.5)	4868 (97.6)
Pennsylvania	6469	1181 (18.3)	1140 (17.6)	196 (16.6)	766 (64.9)
Totals	21,300	6622 (31.1)	5958 (28)	5466 (82.5)	6082 (91.8)

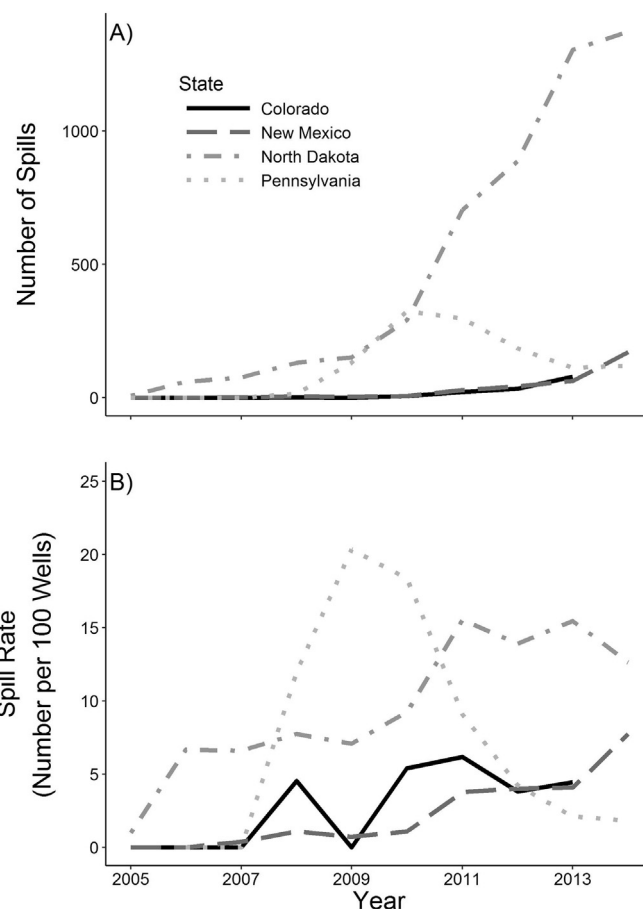


Fig. 1. Number of identified unconventional oil and gas spills (A) and number of spills per 100 wells spudded cumulatively since 1995 (B) in Colorado, New Mexico, North Dakota and Pennsylvania from 01 January 2005 through 31 December 2014. Note: numbers do not denote unique spills because we considered spill reports with more than one material as separate spills. Data for both graphs are located in Table S2.

in the top three spilled materials for all states (excluding unknowns for PA) and crude oil in the top two spilled materials for CO, NM and ND (Fig. 2A, Table S3). Top spilled material rates per 100 spudded wells consisted of crude oil (2.7% of wells), drilling waste (2.5%), wastewater (1.7%) and HF solution (0.5%) in CO; wastewater (6.3%), crude oil (5.5%), natural gas (1.3%) and drilling waste (0.5%) in NM; crude oil (24.1%), wastewater (14.1%), drilling waste (3.2%) and HF solution (1.4%) in ND; and unknown (6.4%), drilling waste (3.6%), wastewater (3.3%) and natural gas (1.9%) in PA (Table S3, Fig. 2B). A total of 5466 spills (82.5%) had reported volumes ranging from 0.4 to 3,752,100 L (Table S4; mean 7119 L, median 757 L). Volumes of the four most frequently spill materials (wastewater, crude oil, drilling waste, and HF fluid) largely ranged from 100 to 10,000 L (Fig. 3). Except for condensate, the largest-volume spills of a specified material all occurred in ND (Fig. 3, Table S4). While freshwater spills were uncommon ($n = 57$

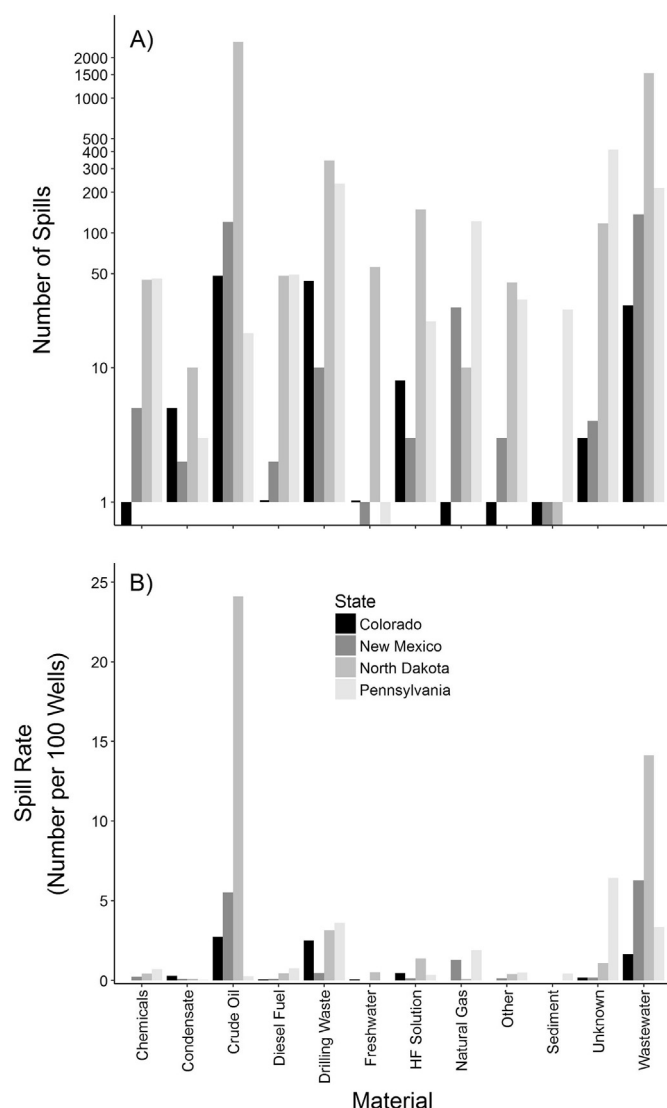


Fig. 2. Number of spills by material for each state (A) and number of spills by material per 100 wells spudded cumulatively since 1995 (B). "Other" includes all materials not included in the 10 listed, and "Unknown" indicates no material was listed in the spill reports. We note that data on the y-axis in panel A are presented on a log scale for display purposes. In panel A, bars below 1 line indicate no spills. Data for both graphs are located in Table S3.

total, 56 in ND, Table S3), their median volume (7949 L) exceeded the median volume of all other spills (715 L) by >10 fold.

3.3. Potential risk to people and nature

Across all states the average distance from a spill to a stream was 580 m and ranged from a low of 0.4 m in PA to a high of 9276 m in NM (Table S5). By state, average distance of spills (from wellhead) to the nearest stream was highest in NM (1379 m), followed by CO (747 m), ND (598 m) and then PA (268 m) (Fig. 4, Table S5). Across all states, 7% of total spills were within 30.5 m (100 ft setback) of a stream, 13.3% were within 61.0 m (200 ft setback), 20.4% were within 91.4 m (300 ft setback), and 47.1% were within 228.6 m (750 ft setback) (Table 2). Pennsylvania has the smallest setback regulation at 30.5 m and 5.3% of the spills in this state were within this distance to a stream. Colorado, NM and PA all had setbacks of 91.4 m and 11.5%, 9.8% and 17.4% of spills in these states, respectively, occurred within this distance from a stream. North Dakota had no specified setbacks from streams,

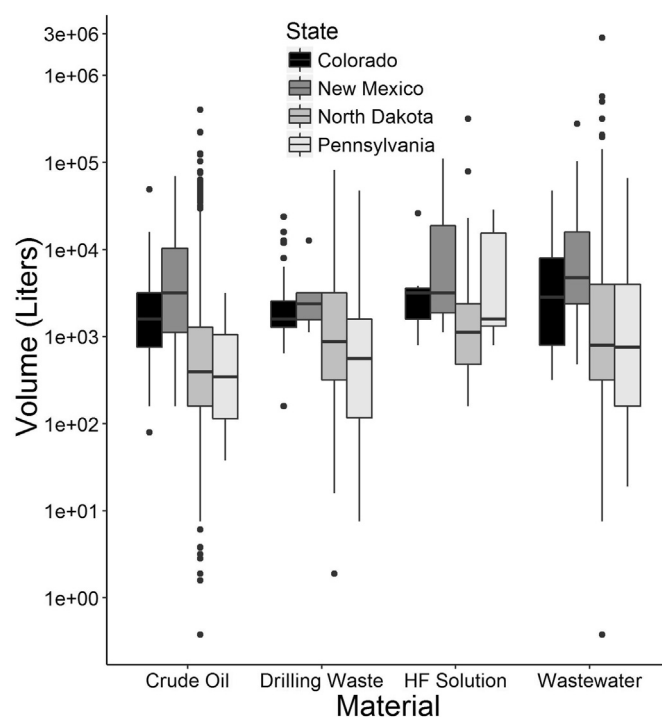


Fig. 3. Volume (L) of spills for the four most often reported spilled materials by state. Box lower and upper hinges correspond to the 1st and 3rd quartiles (25th and 75th percentiles), solid horizontal line are medians, lower whiskers extend from the hinge to the lowest values within 1.5 times the interquartile range (difference between first and third quartiles) of the hinge, the upper whiskers extend from the hinge to the highest value that is within 1.5 times the interquartile range of the hinge, and points beyond whiskers are outliers. We note that data on the y-axis are presented on a log scale for display purposes. Data for graph are located in Table S4.

and other than PA at the 228.6 m distance, had the highest percentage of spills within each setback distance (Table 2).

The overall average index of watershed importance to surface drinking water was highest in PA; PA values were on average 3.2 times higher than CO, 7.5 times higher than NM and 3.8 times higher than ND (Fig. 5A, Table S6). Concurrently, UOG spills also occurred in watersheds with highest importance to surface drinking water in PA (85.1), followed by CO (23.9) and ND (23.0), and lowest in NM (5.8) (Fig. 5B).

4. Discussion

Understanding the characteristics of spills is key to effectively evaluate environmental risk due to UOG development. Risk is a function of the frequency of spills, the type of material spilled, the volume of material spilled, and the proximity of the spill to surface waters and other ecologically sensitive systems. In our study, wastewater and crude oil were two of the most frequently spilled materials across all states, which is consistent with previous reports (COGCC, 2014; USEPA, 2015a). Further, a large subset of spills occurred within current setback regulation distances, which suggest some risk to streams is occurring within these distances. Spills also occurred in watersheds highly important to surface drinking water especially in PA, inferring this state's freshwater resources for drinking may be at higher risk.

The prevalence of wastewater and crude oil spills is likely a result of the large amount of both materials produced, stored and transported (Maloney and Yoxtheimer, 2012; Kondash and Vengosh, 2015). Wastewater is often high in salinity, toxic trace elements, naturally occurring radioactive materials, and other constituents depending on the producing formation and fluid mix involved in fracturing (Rowan et al., 2014; Lauer et al., 2016). Exposure to wastewater has been shown to increase plant mortality of terrestrial plants (Adams, 2011), reduce juvenile mussel survival rates (Patnode et al., 2015), and lower streambed

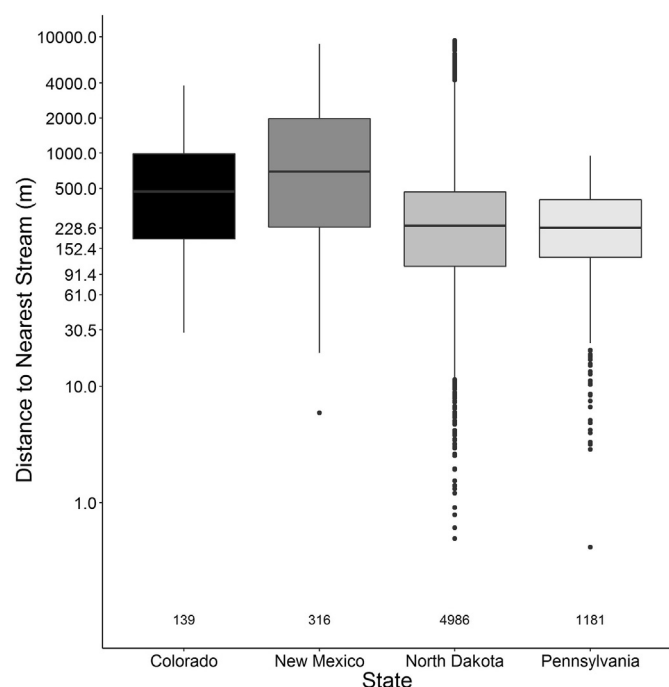


Fig. 4. Distance of spills to the nearest stream (NHDplus high resolution flowline) by state. Numbers below boxplots signify sample size. Box lower and upper hinges correspond to the 1st and 3rd quartiles (25th and 75th percentiles), solid horizontal line are medians, lower whiskers extend from the hinge to the lowest values within 1.5 times the interquartile range (difference between first and third quartiles) of the hinge, the upper whiskers extend from the hinge to the highest value that is within 1.5 times the interquartile range of the hinge, and points beyond whiskers are outliers. We note that data on the y-axis are presented on a log scale for display purposes, and we included common setback distances discussed in text. Data for graph are located in Table S5.

microbial diversity (Akob et al., 2016). Modeling and field studies suggest chemicals associated with wastewater spills can persist in the environment for several years (Rogers et al., 2015; Lauer et al., 2016). Similarly, crude oil spills can have long-term environmental impacts due to the tendency of waterway sediments to adsorb and retain oil's hydrophobic constituents (NASEM, 2016). Besides direct mortality and alterations of community structures, crude oil toxins can negatively affect individuals by causing genetic damage, enzymatic and hormonal changes, immuno-suppression, and bioenergetics and behavioral alterations (NASEM, 2016; Perhar and Arhonditsis, 2014).

Other materials that were spilled less frequently, including hydraulic fracturing fluids (HF solution) and drilling waste, may also pose environmental risks. Nearly 1000 chemicals have been used in various HF solution formulations (Konschnik and Dayalu, 2016); only a small subset of chemicals are used for each individual well and the sum of these additive ingredients is frequently only 1–2% by mass of the total HF solution (USEPA, 2015b). Nevertheless, the large volume of HF solution used in the fracturing process (Kondash and Vengosh, 2015) increases

Table 2

Number of spills within specified distance of streams corresponding to existing setback regulations. Numbers in parentheses indicate the percentage of spills within the setback distance out of total spills (see total column in Table 1). Grey shading represent setback distances specified for each state depending on regulation, where North Dakota had no setback distances specified.

State	Distance (m)				
	30.5	61.0	91.4	152.4	228.6
Colorado	2 (1.4)	7 (5.0)	16 (11.5)	28 (20.1)	41 (29.5)
New Mexico	13 (4.1)	25 (7.9)	31 (9.8)	47 (14.9)	78 (24.7)
North Dakota	388 (7.8)	736 (14.8)	1095 (22.0)	1722 (34.5)	2410 (48.3)
Pennsylvania	63 (5.3)	116 (9.8)	206 (17.4)	378 (32.0)	589 (49.9)
Totals	466 (7.0)	884 (13.3)	1348 (20.4)	2175 (32.8)	3118 (47.1)

the potential for release of these chemicals to the environment. Moreover, many of the chemicals most frequently used are hydrocarbons such as light petroleum distillates (Konschnik and Dayalu, 2016), which can persist in the environment. Drilling waste, a composite of drill cutting, mud, and fluids, was mostly reported in ND and PA. These wastes, especially the cuttings, could contain low levels of naturally-occurring radioactive materials and other constituents, such as trace metals (Johnson and Graney, 2015). Finally, it is interesting to note that while not necessarily as frequent, freshwater spills were on average much larger than other spills, suggesting that frequency alone may underestimate the potential effects of spills. Potential ecological effects from a freshwater spill differ from the other materials and could include increased erosion and sedimentation.

This is the first report we are aware of that reports on proximity of spills to streams. Spills in all states occurred within their smallest setback distances from streams (30.5 m in PA; 61.0 m in NM; and 91.4 m in CO). It is possible that the close proximity of these spills to streams was the result of using a dataset that contained intermittent and ephemeral streams. However, our goal was to identify any possible conduit to a stream, even if a non-flowing system, hence the close proximity to spills indicates a potential for spilled material to reach larger streams. The number of spills within setbacks also could be because we identified the spatial position of a spill using the coordinates of the wellhead rather the supporting equipment such as flowlines or storage tanks, however as indicated before, we have no reason to believe a directional bias in over- or under-estimating spill distances. Finally, the number of spills within setbacks could be a result of waivers in some state codes (e.g., 58 Pa. Stat. § 3215(b)) that allow construction of wells within the setback; however data to evaluate this were not available for our study; a future analysis could focus on wells that were granted waivers or exemptions.

We recognize that other factors, in addition to proximity, influence the vulnerability of streams to spills. Spilled fluids may reach streams by overland flow or following infiltration and flow along subsurface pathways. The vulnerability of streams to spills that move via the subsurface track will depend, in part, on proximity, but will also reflect hydrogeologic properties that govern water-table depth, the rate and directions of groundwater flow, and whether the stream gains water from, or loses water to, the groundwater reservoir. The site-specific nature of these properties implies that assessment of threats that spills pose to stream-water quality must be conducted on a case-by-case basis.

Ecosystems provide many benefits to people, including food and water, regulating floods and diseases, and recreation (Millennium Ecosystem Assessment, 2005). We evaluated the potential effects of UOG spills on ecosystem services by focusing on surface drinking water, using an importance index developed by the US Forest Service. We found that spills in Pennsylvania occurred in watersheds with a much higher value to surface water than the other states, a result of higher population density and reliance on surface waters as drinking water in this area (Weidner and Todd, 2011). The other three states had spills in watersheds with lower importance values, which likely reflects a greater reliance on groundwater or that drinking water is sourced from streams and reservoirs of distal watersheds. Nevertheless, in these states a localized spill that affects water reservoirs and treatment plants can negatively impact communities near UOG development.

Several approaches to model the risk of UOG development have been recently implemented, from probability modeling (Rozell and Reaven, 2012) to expert risk assessments (Walker et al., 2016). Our results suggest that modeling the risk of hydraulic fracturing will require region-specific parameterizations. For example, North Dakota had the highest spill rates for both crude oil and wastewater as well as the largest reported spill volumes for these materials. The Bakken is the largest producer of crude oil (USEIA, 2016) and wastewater (Kondash and Vengosh, 2015) for plays in our study, which could be one reason for

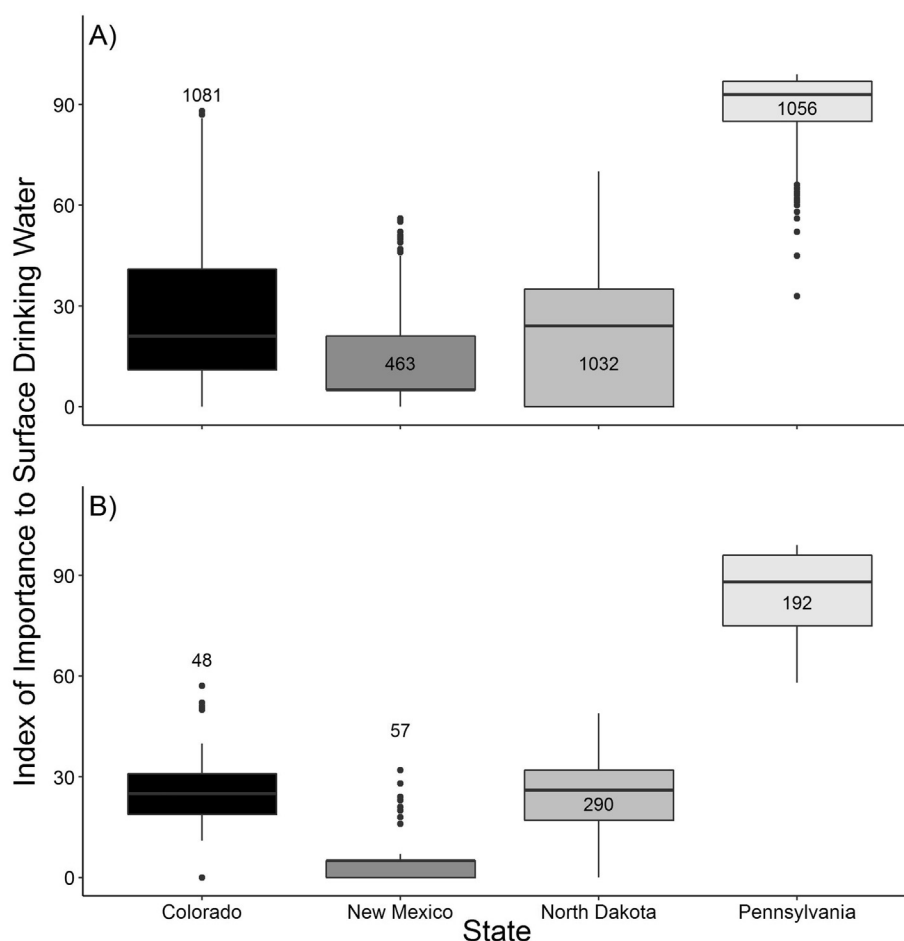


Fig. 5. Forest to Faucet index of importance to surface drinking water for all HUC12 watersheds in the Forest to Faucet data set in each state (A) and for the subset of these HUC12 watersheds in which a UOG spill occurred (B). Number within or above boxplots are sample size. Box lower and upper hinges correspond to the 1st and 3rd quartiles (25th and 75th percentiles), solid horizontal line are medians, lower whiskers extend from the hinge to the lowest values within 1.5 times the interquartile range (difference between first and third quartiles) of the hinge, the upper whiskers extend from the hinge to the highest value that is within 1.5 times the interquartile range of the hinge, and points beyond whiskers are outliers. Data for graph are located in Table S6.

the higher spill rates. Another reason may be development rate, where ND had over twice the number of spudded horizontal wells in 2012, 2013 and 2014 than the other three states. Finally, differences in reporting are also a factor; ND requires reporting at a lower threshold (1 barrel; 159 L) than either CO or NM (5 bbls, 795 L), and its dataset also had the most complete records in terms of materials and volumes associated with spills. Unifying reporting requirements across states would aid future broad-scale data analyses and risk modeling.

Finally, we acknowledge that all spills may not have been reported or included in the available state databases. Data were not available to estimate the frequency of these “missing” spills and we are unaware of any study that has directly examined missing spills (but see Wiseman, 2013). To deter under-reporting, states in our study have penalties for failing to report a required spill that include maximum civil penalties that range across the states from \$12,500 (ND) to \$15,000 (CO) per day to \$75,000 plus \$5000 per day (PA); NM, ND, and PA also categorize a failure to report as a possible criminal act (Colorado House Bill 14–1356, Section 1, 34–60–121; North Dakota Century Code Section 38–08–16.1; New Mexico House Bill 286 Section 70–2–31(B); 58 Pa State § 3255, 3256). Further, our large sample size ($n = 6622$) from four states over ten years likely averages any effects of under- or over-reporting, and missing spills are thus not likely to affect overall patterns.

5. Conclusions

Concerns over potential environmental issues resulting from UOG development have spurred a flurry of recent articles on its potential ecological effects to associated ecosystems (Brittingham et al., 2014; Evans and Kiesecker, 2014; Souther et al., 2014). An important gap in our understanding of potential effects of UOG on associated ecosystems is the surface release of chemicals, waste materials and oil and gas. We found that wastewater, crude oil, drilling waste and hydraulic fracturing solution are the materials most often involved in a spill incident, and that most spills ranged between 100 L and 10,000 L. We also found that some spills occurred in very close proximity to streams and in watersheds of high importance to drinking water. Further, spills occurred within current setback requirements. As UOG activity continues in the U.S. and commences in other countries around the world, our findings can be used to better understand the risk of spills from UOG development and help inform management decisions, policy, and regulations.

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Competing financial interest declaration

The authors declare no competing financial interests.

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High volume hydraulic fracturing operations: potential impacts on surface water and human health

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ABSTRACT

High volume, hydraulic fracturing (HVHF) processes, used to extract natural gas and oil from underground shale deposits, pose many potential hazards to the environment and human health. HVHF can negatively affect the environment by contaminating soil, water, and air matrices with potential pollutants. Due to the relatively novel nature of the process, hazards to surface waters and human health are not well known. The purpose of this article is to link the impacts of HVHF operations on surface water integrity, with human health consequences. Surface water contamination risks include: increased structural failure rates of unconventional wells, issues with wastewater treatment, and accidental discharge of contaminated fluids. Human health risks associated with exposure to surface water contaminated with HVHF chemicals include increased cancer risk and turbidity of water, leading to increased pathogen survival time. Future research should focus on modeling contamination spread throughout the environment, and minimizing occupational exposure to harmful chemicals.

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Introduction

High volume, hydraulic fracturing (HVHF), or known by industry jargon as “fracking”, is a process used to collect natural gas and oil from deep, underground shale deposits (US EPA 2015a). While the use of HVHF to optimize extraction of oil and natural gas was first patented in 1869 (USPO 1869), the relatively novel use of HVHF in horizontal wells began in the 1990s, only making a move to the eastern states (Pennsylvania) in 2003. This relatively novel approach increased the technique’s yield potential, economic viability, and popularity. With the use of horizontal drilling, and subsequent injection of a mixture of water, sand, and chemicals, large amounts of natural gas and oil can be efficiently extracted from previously inaccessible, impermeable shale deposits. HVHF operations involve drilling vertically to a specified depth, which depends on the properties of each well, followed by the drilling of a horizontal borehole into the shale formation. The borehole is then prepped for gas extraction and filled with a proprietary mixture of chemicals, water, and sand. This mixture serves several purposes: the sand acts to keep fractures open (proppant) in order for the natural gas to escape freely; the large variety of chemicals each serve a specific purpose, e.g. glutaraldehyde as a biocide to keep biofilms from forming and clogging up pores in the well casing, others (isopropanol, hydrochloric acid, and ethylene glycol) are used in the process to increase efficiency and yield (US EPA 2011); additional chemicals and their purpose are found in Table 1. Due to the dangerous properties of these incorporated chemicals,

there has been increased concern over their accidental introduction into drinking, and recreational, water sources and their effects on human health (US EPA 2010).

As of 2014, the USA is the worldwide leader, with large HVHF operations being used to extract oil and natural gas from shale found in Pennsylvania, Texas, Colorado, and the Dakotas (Table 2). These areas contain substantial shale deposits and have numerous HVHF wells. Permits to use the HVHF process have also been issued internationally. In Europe, Poland and the UK have all begun HVHF operations with first attempts already completed (McBride & Sergie 2015). Outside of Europe, China hopes to soon begin shale development, as it sits atop the world's largest natural gas shale deposits (Figure 1). However, water shortages and terrain difficulties complicate the extraction process (Fensom 2014). The relatively new HVHF process is of concern because the many nations that are interested, or already involved, in HVHF have loose environmental policies and, in some cases, little oversight of industry practices has been provided. Furthermore, human and ecological health consequences associated with HVHF operations have not been well researched.

Due to the contamination potential of HVHF operations, loose regulation of industry practices (US EPA 2014c), and global interest in this relatively novel industry (Fensom 2014), it is important to explore how environmental matrices will be impacted by further growth in the use of HVHF to exploit shale energy. This is especially true for water sources used for drinking water, recreation, and aquaculture. Although various studies have focused on groundwater contamination as a result of HVHF operations, not enough emphasis has been placed on surface water impacts. Additionally, because human health is so closely linked to water quality and environmental integrity, we choose to focus on various aspects of hydraulic fracturing operations that may alter the integrity of bodies of

Table 1. An example of the volumetric composition of hydraulic fracturing fluid. Although fluid contents are proprietary, and usually not disclosed.

Component/ additive type	Example compounds	Purpose	Percent composition (by volume)	Volume of chemical (gallons) ^a
Water		Deliver Proppant	90	2,700,000
Proppant	Silica, quartz sand	Keep fractures open to allow gas flow out	9.51	285,300
Acid	Hydrochloric Acid	Dissolve minerals, initiate cracks in rock	0.123	3690
Friction Reducer	Polyacrylamide, mineral oil	Minimize friction between fluid and the pipe	0.088	2640
Surfactant	Isopropanol	Increase the viscosity of the fluid	0.058	2550
Potassium Chloride		Create a brine carrier fluid	0.06	1800
Gelling Agent	Guar gum, Hydrox- ethyl Cellulose	Thicken the fluid to suspend the proppant	0.056	1680
Scale Inhibitor	Ethylene Glycol	Prevent scale deposits in the pipe	0.043	1290
pH Adjusting Agent	Sodium/Potassium Carbonate	Maintain effectiveness of other components	0.011	330
Breaker	Ammonium Persulfate	Allow delayed break- down of the gel	0.01	300
Crosslinker	Borate Salts	Maintain fluid viscosity as temperature increases	0.007	210
Iron control	Citric Acid	Prevent precipitation of metal oxides	0.004	120
Corrosion Inhibitor	N,N-dimethyl For- mamide	Prevent pipe corrosion	0.002	60
Biocide	Glutaraldehyde	Eliminate bacteria	0.001	30

Notes: These data were gathered by the US Environmental Protection Agency (2011). The original table has been altered in order to best fit formatting, and publication specifications.

^aBased on 3 million gallons of fluid used.

Table 2. An estimate of fracking (HVHF) wells by state, as of October 2013. Number of fracking wells per state as reported after 2005. More specific data is provided for 2012 in order to illustrate the growing prevalence of hydraulic fracturing in each state.

State	Fracking wells since 2005	Fracking wells drilled in 2012
Arkansas	4910	719
Colorado	18,168	1896
Kansas	407	236
Louisiana	2327	139
Mississippi	9	Unavailable ^a
Montana	264	174
New Mexico	1353	482
North Dakota	5166	1713
Ohio	334	234
Oklahoma	2694	Unavailable ^a
Pennsylvania	6651	1349
Tennessee	30	Unavailable ^a
Texas	33,753	13,540
Utah	1336	765
Virginia	95	1
West Virginia	3275 ^b	610 ^b
Wyoming	1126	468
Total	81,898	22,326

Notes: Original table by Environment America Research and Policy Center was altered, with permission, to better fit formatting (Ridlington & Rumpler 2013).

^aUnavailable means information was not available to determine when wells were drilled.

^bData for West Virginia are for permitted fracking wells, not wells that have been drilled. Data were not available on drilled wells.



Figure 1. (Color online) World map of assessed shale resources. Data compiled illustrating assessed shale formations throughout the world, with depiction of whether resource estimates are available. Estimates place China as the holder of the largest shale resources, with the United States second. Map by US Energy Information Administration (US EIA 2013). Legend altered for better resolution.

water and the environment. The goal of this article is to compile information from existing scientific works, with an aim to identify current and future impacts of HVHF operations that have the potential to threaten human health by impacting surface water quality.

Methodology

Information on hydraulic fracturing was gathered using multiple search engines in order to most efficiently obtain reliable sources of information. These search engines include: Google Scholar, JOSTR,

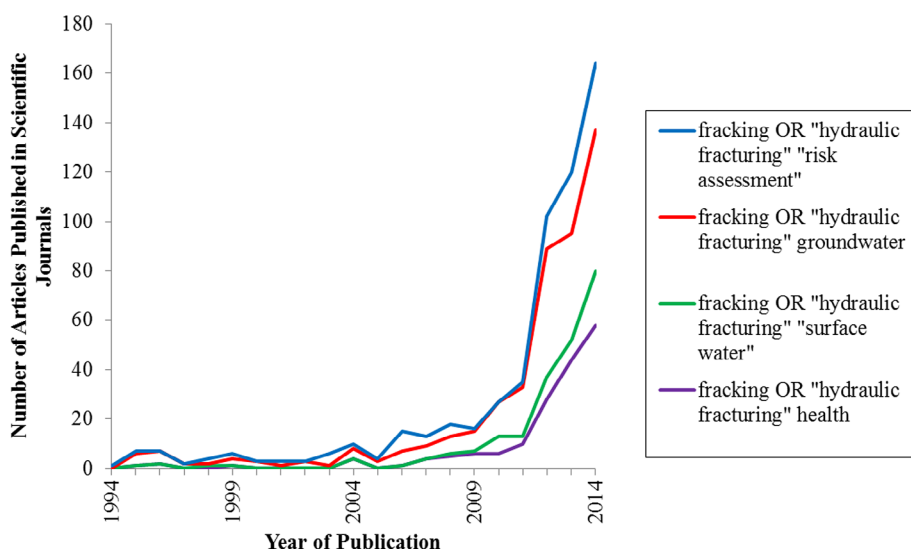


Figure 2. (Color online) Hydraulic fracturing themed scientific journal publications per year, over a 20-year period (1994–2014). Number of hydraulic fracturing themed studies, graphed by search terms used to attain results from Scopus (Elsevier B.V. 2015) searches. Publications were limited to a 20-year period starting in 1994 in order to account for recent advancements in the HVHF process. Data are current as of 28 March 2015.

Science Direct, and Scopus. The Google Scholar search engine was used to search for general information on hydraulic fracturing, and to cross-reference multiple sources of articles. Searches were conducted for the terms “hydraulic fracturing worldwide” and “hydraulic fracturing process.” Initially, results were limited to “.gov” and “.org” websites in order to eliminate potentially biased sources. Later, searches were expanded to include all domain names, with special care to avoid publications with potential conflict of interest. Reliability of sources was the most crucial determinant when selecting websites and articles. Mostly, but not exclusively, government and scientific journal articles were used to ensure credibility.

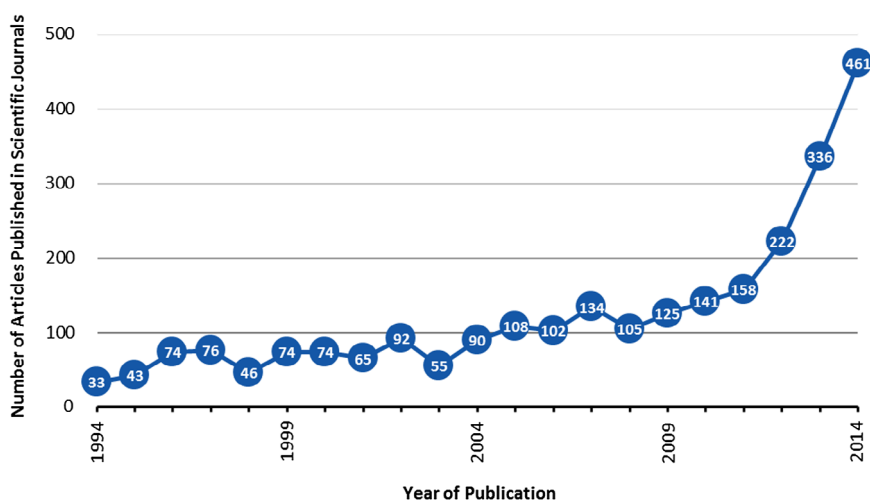


Figure 3. Number of hydraulic fracturing themed articles published per year. A graphical depiction of the sharp increase in hydraulic fracturing research over a 20-year period, beginning in 1994. Recent economic conditions have made HVHF a viable option for resource extraction, increasing its prevalence worldwide. With an increase in prevalence, there is also an increase in studies exploring the practice. Data were gathered using Scopus search (Elsevier B.V. 2015), and are current as of 28 March 2015.

Other search engines, such as Science Direct and JOSTR, were used to find relevant studies, reports, and articles. Along with Google Scholar, Science Direct and JOSTR were searched using the keywords “hydraulic fracturing surface water,” “hydraulic fracturing wastewater,” and “hydraulic fracturing ‘risk assessment.’” The results of these searches were used in writing this article, along with several websites, reports, and findings from the US Environmental Protection Agency.

Additionally, the mentioned search engines were used to help pinpoint gaps in information and studies of the hydraulic fracturing process. Upon conducting multiple searches, there was an evident lack of information on the impact of hydraulic fracturing on human health due to alteration of surface water systems. This gap was also outlined by a Scopus (Elsevier B.V. 2015) search result analysis, with data current as of 28 March 2015 (Figure 2). This analysis showed that although, overall, studies dealing with HVHF have significantly increased over time (Figure 3), not enough emphasis is placed on environmental and human health impacts (Figure 4).

In order to find data, articles, and reports to address the gap in literature, we had to keep bias in mind at all times. Due to the polarizing and divisive nature of the issue addressed, articles and reports were chosen in a careful manner that avoided those backed by industry interests, and “anti-fracking” organizations. In this way, we hoped to avoid bias and preserve neutrality in our presentation of data. In all 24 reports were cited, the majority of which were funded, or published, by the US Environmental Protection Agency (US EPA), with others coming from organizations such as the World Health Organization (WHO), and the World Resources Institute (WRI). In addition to these reports, 23 scientific articles were referenced, from publications such as PNAS, Nature, Toxicology, Risk Analysis, etc. Other information, such as chemical properties was found on the US EPA’s Integrated Risk Information System (IRIS), and the US EPA website. Through the use of widely trusted sources

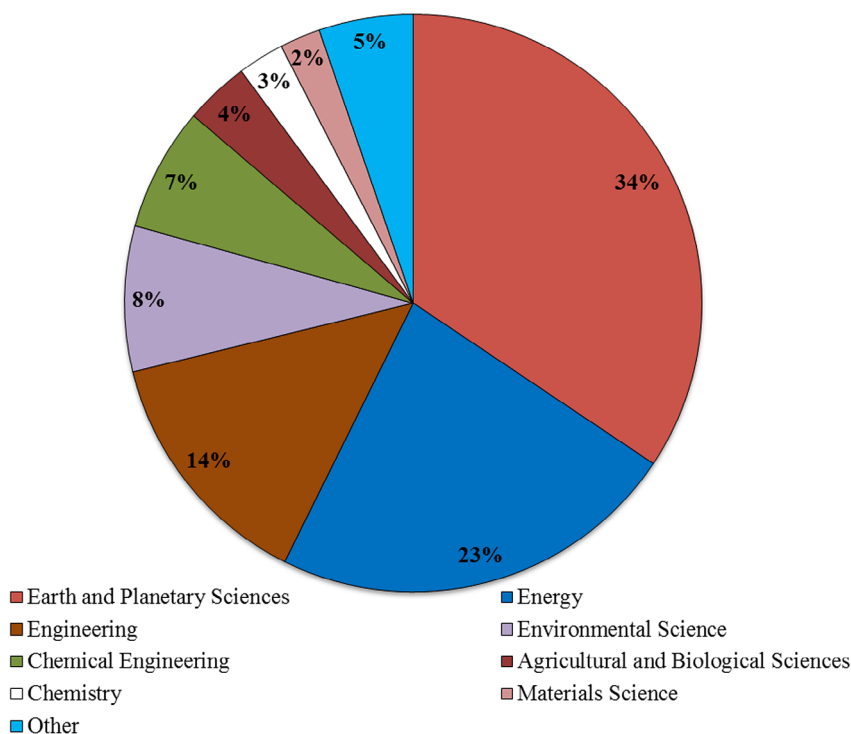


Figure 4. (Color online) Percent breakdown of subject matter of 2614 articles dealing with hydraulic fracturing. A Scopus (Elsevier B.V. 2015) search was done for the term “hydraulic fracturing OR fracking” with articles dealing with economics, computer design, business, etc. being filtered out of results. Results were also confined to a 20-year span, beginning in 1994, to match previous data analysis. Search data are current as of 28 March 2015.

and avoidance of publications with potential conflicts of interest, we believe we have avoided the use of potentially biased works.

General hazards associated with HVHF

There are multiple hazards associated with natural gas and oil. One such hazard is air pollution, due to the nature of the procedure, where harmful chemicals, such as benzenes and xylenes, are released into the air. Exposure to these chemicals may cause irritation and possibly contribute to an increased risk of cancer (IRIS 2000, 2014). Due to the imperfect nature of the gas collection process, 3.6 to 7.9 % of methane will escape into the atmosphere during the lifetime of the well (Howarth et al. 2011). The potential contribution of methane to the greenhouse effect must be given research priority (US EPA 2014b), as several studies have found that methane has a significantly higher potential to trap heat over its lifetime, than CO₂ (Howarth et al. 2011; Yvon-Durocher et al. 2014; US EPA 2014b).

HVHF also introduces the issue of terrain alteration to make way for necessary infrastructure (Figure 5). It has been known that each HVHF well requires about 3.03 hectares of cleared land, with most actually needing about 7 to 8 hectares (Johnson 2010; Entekin et al. 2011). This amount not only includes the pad itself, but waste pit areas, access roads, facilities, and other infrastructure. Therefore, habitat degradation along with ecosystem disruption and landscape alteration are of real concern. With the alteration of terrain to build new roads and other HVHF-related infrastructure in the area, soil erosion rates increase. Williams et al. (2007) have demonstrated that this increased erosion affects nearby water bodies (discussed in more detail later in this article).

Along with increased rates of erosion, removal of water from the water cycle can negatively impact surrounding areas. A large volume of the water used in HVHF is not recovered, too polluted to be discharged safely, unfit for reuse, or polluted to a point beyond effective treatment; therefore, it must be injected into deep disposal wells (Hammer & VanBriesen 2012). Over time, this process removes billions of liters of water from the water cycle depending on well density in the area (US EPA 2011). Examples of this include counties found in the Eagle Ford, Haynesville, and Barnett Shales, where 38, 11, and 18 % of ground water was used for the purposes of unconventional energy extraction (Jackson et al. 2014). This is of special concern when looking at shale formation locations. Around the world, 38 % of shale is found in water-stressed or arid regions; areas where 40–100 % of fresh water resources are already allocated to other uses (Reig et al. 2014) and are home to 386 million people. Due to the remote locations of these wells, water stress in these areas can be of great concern (Reig et al. 2014), especially when millions of liters, per well are withdrawn for HVHF (Figure 6).



Figure 5. Two HVHF well pads near Beaver Run Reservoir, Westmorland County, PA. Beaver Run Reservoir supplies three townships, home to 150,000 people. Although fishing is banned, the Municipal Authority of Westmorland County has allowed drilling companies to extract natural gas from near the reservoir, using hydraulic fracturing. Photo used with permission from marcellus-shale.us (2010).

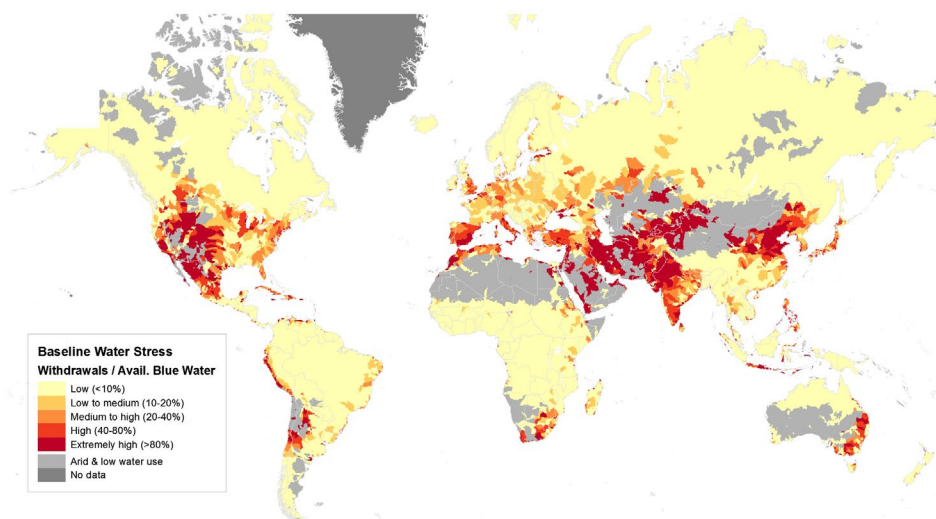


Figure 6. (Color online) Map of baseline water stress throughout the world. Baseline water stress was calculated as a ratio of water withdrawal and available blue water. Data show highly stressed areas overlap with 38 % of shale resources. Potential HVHF in stressed areas, home to 386 million people, could further decrease available water resources. Map was generated using Esri ArcMap10 software (Esri 2014), and data from the World Resources Institute (WRI 2013), in accordance to their public use policy.

Nearby contaminated groundwater may infiltrate into failing household wells; e.g. previous studies have shown that methane permeates aquifers and pollutes well water (Osborn et al. 2011). Although not harmful to humans when consumed directly, methane can cause dizziness, headache, and other health issues when inhaled (US EPA 2014b). Combined with the flammable properties of methane, it is imperative that incidence of faulty wells be minimized.

Surface water risks attributed to HVHF

Relative risk and failure rates associated with wells in Pennsylvania

One of the less-addressed concerns associated with the HVHF process is its impact on surface water systems, with few studies addressing the issue. As millions of people rely on surface waters systems for as a source of drinking water, contamination could potentially endanger the population nearby. Containment failures, illegal dumping, and accidents during transport of untreated water pose potential threats to surface waters (Hunt 2013). Due to the enormous volume of water used, remote location of wells, complexity of the HVHF procedure, and the 3950 truck trips needed during the early development phases, water systems are put under an increased risk of contamination (New York State Department of Environmental Conservation (NYSDEC) 2011). Special concerns are raised by the amount of traffic needed to properly conduct a HVHF operation, leading to an increased potential for accidents and spills into streams, rivers, and other bodies of water (NYSDEC 2011). A majority of these concerns, including acquisition of water, transport, treatment, and disposal of waste, are echoed by Rodriguez and Soeder (2015). Rodriguez and Soeder also explored the impact of hydraulic fracturing on surface water quality, but did not focus on the implications these impacts have on human health. We choose to further expand on this area of information by also addressing human health consequences of surface water contamination.

Along with increased traffic, Ingraffea et al. (2014) state that wells can fail due to structural integrity issues. As horizontal wells are drilled for shale exploitation, and prepped for HVHF, steel casings are set to prevent liquids and gasses from entering underground aquifers and, subsequently, contaminating groundwater. Improper construction, or assembly, and other factors can lead to loss of integrity,

causing casings to leak. Ingraffea et al. also state possible reasons for cement failure, e.g. inappropriate cement density, inadequately cleaned bore holes, cement shrinkage, and premature gelling of cement (2014). Additionally, a study by Darrah et al. (2014), also found that faulty well construction contributed to contamination of drinking water wells in the Marcellus, as well as the Barnett, shale regions. This demonstrates that the issue of faulty well construction leading to leaks is not specific to the Marcellus region.

Data, collected by Ingraffea et al. (2014), show that incidence rates of cement and casing failures in unconventional wells are up to six times higher than that found in conventionally operated wells in Pennsylvania. Pre-2009 conventional wells were found to have a failure rate of 5.21 %, compared to 9.84 % in unconventional wells. It should be noted in this study, that failure rates in unconventional wells only reflects a small sample size (61 wells) and are not valid indicators of positive or negative aspects of current methods and precautions taken during current well construction. After 2009, conventional well safety increased in the Northeastern counties of Pennsylvania, resulting in a reported failure rate of 2.27 %, a drop from the previous 5.21 %, while the failure rate of unconventional wells largely remained the same (a 9.14 % failure rate) (Ingraffea et al. 2014).

This is similar to the findings reported by Jackson et al. (2014), who studied well integrity in Canada. Jackson et al. (2014) used gas migration and sustained casing pressure to evaluate well integrity. They noted that horizontal wells were three, to four, times more likely to experience gas migration and sustained casing pressure than vertical wells.

Although tempting, one cannot compare conditions before and after 2009, because older wells are more likely to fail and have had a longer history of inspections, thus allowing early failures to appear on record. According to Ingraffea et al. (2014), well age is critical to observing incidence of failure. However, it should be stated that, in NE Pennsylvania, unconventional wells were at 2.7 times the relative risk to fail structurally, than conventional wells. Finally, all wells, both conventional and unconventional, in the NE counties were 8.5 times more likely to experience structural failure, than those drilled outside of the area.

Taking this information into account, a recent US EPA (2015c) retrospective study, has concluded that there is no association between HVHF and well water contamination. This study contradicts previous hypothesis, much like the one presented by Ingraffea et al. (2014), that HVHF wells are more likely to contribute to groundwater contamination than conventional wells. However, even though no evidence was found of well water intrusion by HVHF chemicals in the Bakken, Barnett, and Raton Basins, the study did discover high chloride levels in groundwater near a HVHF well impoundment site in Southwestern Pennsylvania (US EPA 2015c). Additionally, the study noted that a pond site studied in Northeast Pennsylvania also had elevated chloride levels, as well as total dissolved solids (TDS) (US EPA 2015c). This pond was not used for drinking water purposes, but the situation demonstrates the existing hazard to surface water contamination from HVHF. A summary of key findings from the US EPA HVHF study can be found in Figure 7 and Table 3.

Wastewater treatment risks

HVHF-related processes also have the potential to deteriorate water quality via improper wastewater treatment and discharge of produced water (a byproduct from shale fracturing that is captured, stored, and then shipped for treatment to nearby facilities) (Wilson & VanBriesen 2012). In Pennsylvania, companies were allowed to take produced water to publically owned wastewater treatment plants (POTWs) or centralized brine plants (CWTs) (Wilson & VanBriesen 2012). These treatment plants removed constituents from produced water and brine, but failed to address the problem of salts, leading to discharges from plants carrying significant levels of total dissolved salts and bromide (Wilson & VanBriesen 2012).

In some cases, POTWs and CWTs were granted exemption from discharge limits on dissolved solids. This exemption resulted in an increase of salt load to surface waters, as well as introduction of bromide into drinking water sources (Wilson & VanBriesen 2012). Shale rock formations are notoriously

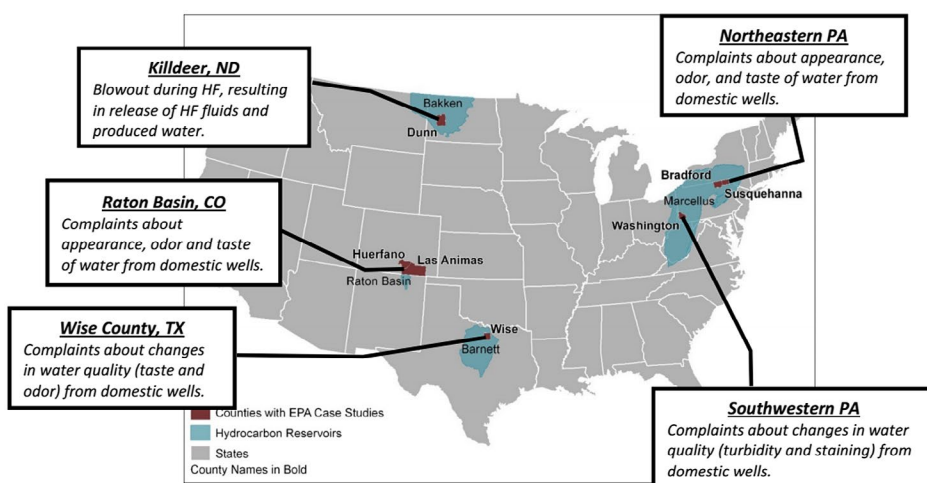


Figure 7. (Color online) Study areas, and justification for choosing each area of study, in a US EPA retrospective case study on HVHF contamination of water supplies. This figure shows the areas of the USA chosen to be involved in the retrospective study, and the reasons each area was chosen. Areas were chosen in multiple, major shale formations, and narrowed down for sampling to determine if contamination had taken place.

high in bromide content. They contain, on average, $24,000 \mu\text{g L}^{-1}$ bromide, unlike other rock types that hold an average of $6000 \mu\text{g L}^{-1}$ (Bowen 1966). This led to the situation described by Wilson & VanBriesen, as well as Hladik et al. (2014) who reached a similar conclusion, stating that POTWs and CWTs treating hydraulic fracturing wastewater introduced disinfection byproducts (DBPs), and DBP precursors, to streams into which treated water was discharged. While sampling near POTWs showed six DBPs, CWTs introduced only two, one of which was dibromochloronitromethane, found at a relatively high level of $8.5 \mu\text{g L}^{-1}$ (Hladik et al. 2014). Additionally, bromide was found at a concentration of $75,000 \mu\text{g L}^{-1}$, compared to mean values of $200 \mu\text{g L}^{-1}$ bromide in freshwater (Bowen 1966).

The introduction of additional bromide to surface water systems is of great concern as brominated water can be toxic, and can result in dangerous brominated DBPs (US EPA 2013b). Such is the case in the formation of bromate, a byproduct of using ozone to treat water which contains bromide (WHO 2005). This becomes an emerging concern for drinking water treatment plants that were designed and optimized for ozone disinfection based on the safe level of bromide in their source water, but now the impacted surface source water can have unsafe levels of bromide. Bromate is a carcinogenic compound, can cause DNA alterations, and has been shown to be mutagenic in the kidneys of lab rats (Moore & Tao 2006). Controlling bromide levels should be a high priority for all plants that are treating HVHF fluids, as even low levels of bromide in water can lead to carcinogen formation. In Pennsylvania, over a two-year period, certain exemptions were applied to treated water that resulted in excess quantities of bromide into water supplies (Wilson & VanBriesen 2012).

Data gathered by Wilson & VanBriesen (2012) show that during the HVHF boom of 2008 in Pennsylvania, the amount of produced water rose from less than 10 million barrels in 2006, to over 40 million barrels in 2008 and 2009. During these same years, it is estimated that more than 50 % of the TDS in the treated produced water were released to surface water systems; 56 % in 2008 and 57 % in 2009 (Figure 8) (Wilson & VanBriesen 2012). The data also indicated that during this time, low flow conditions were seen throughout Pennsylvania, leading to concentration of the discharged chemicals into low volumes of water, and the possibility that the discharges could have affected drinking water (Table 4).

According to Wilson & VanBriesen (2012), data show that new methods for managing produced water and state agencies urging companies not to use POTWs and CWTs without discharge limits, that the bromide and TDS were considerably lower in 2010 than the levels from 2008 and 2009 (Figures

Table 3. Key findings of a retrospective case study conducted by the US EPA (2015c). These findings illustrate that, even though there was no evidence to conclusively link groundwater contamination with HVHF, surface water and ground water sources that are not used for drinking water, were contaminated. These findings justify concerns raised in terms of surface water contamination, and contamination potential of HVHF associated operations.

Location	Key findings
Killdeer, ND	The drinking water wells sampled did not show the presence of chemicals or brine associated with the blowout. However, two monitoring wells screened in the Killdeer aquifer showed the presence of brine and tert-butyl alcohol (TBA) Based on the data analysis performed, the only potential source consistent with the TBA and brine at the two monitoring wells was the blowout during hydraulic fracturing that occurred in Killdeer, ND
Northeastern PA	Background data showed that methane is naturally occurring in the study area; however, using multiple lines of evidence EPA concluded that up to nine of the 36 drinking water wells are impacted by stray gas (methane and ethane) associated with nearby hydraulic fracturing activities
Southwestern PA	Increased levels of chloride in ground water at locations near an impoundment site which contained hydraulic fracturing wastewaters and drilling waste. The chloride contamination likely originates from the impoundment site based on multiple lines of evidence Background data showed that methane is naturally occurring in this area and was detected in 24 % of the samples collected from domestic wells. The isotopic signature of the methane present in domestic wells was not similar to that of gas produced from the shale being hydraulically fractured
Wise County, TX	In one of the three study areas, two domestic wells were impacted. Based on the screening of potential sources of impacts, brines associated with the specific geological formation were the only source that was consistent with the observed impacts to two of the study wells Screening also identified a third well located at an industrial facility that was potentially impacted by brines and/or landfill leachate
Raton Basin, Co	Background data indicates that dissolved methane is naturally present throughout the Raton Basin and EPA detected it in all samples collected from the domestic wells In one of the sampling areas (Little Creek Field; Huerfano County), gas migration had occurred but cannot be definitively linked to hydraulic fracturing Tertiary Butyl-Alcohol (TBA) was also detected in samples from domestic, monitoring, and production well; however, we were not able to confirm the specific source(s) of the TBA

8 and 9). An increase in recycling of produced water, disposal into deep injection wells, and use of treatment plants with discharge limits, all played a part in decreasing bromide and TDS. Despite a change in regulations to address these issues that lead to a decrease in TDS and bromide in 2010 and 2011, discharges were still significantly higher than those measured during 2006 (the “pre-produced water boom” period) (Wilson & VanBriesen 2012). This study is important when looking at nations interested in future use of HVHF technology, especially those with a history of lax regulations of similar industrial practices, waste management, and disregard for human health in favor of economic development.

A risk assessment, using probability bounds analysis (PBA) by Rozell and Reaven (2012), addressed risks from water pollution due to HVHF operations in the Marcellus shale formation. The PBA found that the biggest concern for water contamination due to HVHF operations was from wastewater disposal and the epistemic uncertainty associated with the process. They state, given a 10 % development of the Marcellus region (40,000 wells), that we should expect to see volumes of contaminated water equivalent to several thousand Olympic-sized swimming pools. This calculation was based on a best-case median risk of 200 m³ of contaminated water release per well, further emphasizing the importance of proper waste treatment.

Contamination potential from accidental discharge

Further hazard, associated with HVHF of shale, is from the potential, accidental discharge of chemicals, byproducts, and sediments into surface water systems. As discussed earlier, unconventional, HVHF, wells are between three to six times more likely to experience structural integrity loss than conventional wells (Ingraffea et al. 2014; Jackson et al. 2014). Additionally, Jackson et al. (2014) found that wells experienced structural integrity loss, of at least one barrier (cement or steel casing) depending on factors such as geology and well construction. Rates of sustained casing pressure (a measure of

Table 4. Chemical constituents of TDS in produced water relevant for drinking water treatment plants.

Chemical constituent	Range reported in produced water from Marcellus shale at 14–90 days after hydraulic fracturing (mg/l) ^a		Range reported in produced water from conventional oil and gas production (mg/l) ^b		Range reported in effluent from brine treatment plants in southwestern Pennsylvania ^c		Drinking water maximum contaminant level (MCL) secondary standards (mg/l) ^d
	Range	Median	Range	Median	Range	Median	
TDS	3010–345,000	157,000	68,000–354,000	215,000	16,600–201,100	130,100	500
Chloride	1670–196,000	98,300	5760–207,000	116,500	1752–96,909	57,250	250
Sulfate	0.2–89.3	0.8	1–850	140	5–1500	667	250
Bromide	15.8–1990	849	94–2240	1010	76.2–8290	1105	–

Notes: Data summarizing chemical composition of produced water and brine, from conventional and Marcellus shale wells. Data and table used with permission from Cambridge Press and Dr Jessica Wilson (Wilson et al. 2012). a. Adapted from Hayes (2009). 16 gas wells in Pennsylvania and 3 in West Virginia. TDS, n = 29; chloride, n = 29; sulfate, n = 29; bromide, n = 29. b. Adapted from Dresel and Rose (2010). 24 gas wells and 15 oil wells in Western Pennsylvania. TDS, n = 16; chloride, n = 39; sulfate, n = 36; bromide, n = 38. c. Adapted from US EPA (2012a). TDS, n = 22; chloride, n = 28; sulfate, n = 26; bromide, n = 30. Data are from laboratory analysis submitted by three brine treatment plants in Southwestern Pennsylvania at the request of the US EPA located in the Allegheny River Basin. d. Secondary standards deal with aesthetic issues rather than human health issues (US EPA 2009).

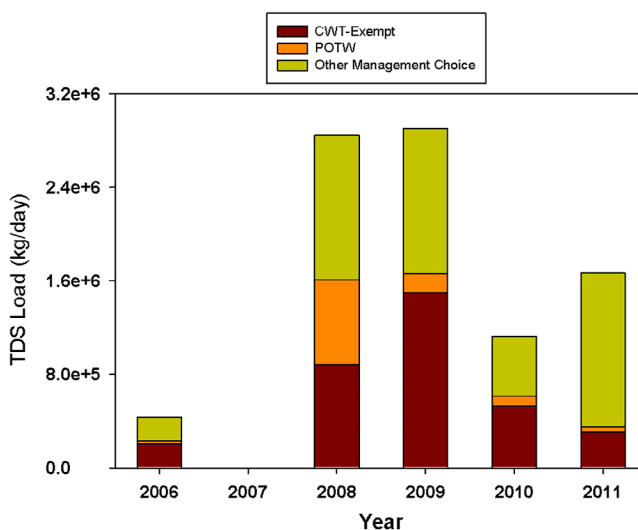


Figure 8. (Color online) TDS loads released into surface waters by Pennsylvania water treatment plants, in kilograms per day, over a five-year period. This Figure shows the trend of TDS loads released in Pennsylvania, demonstrating the impact that CWT's with exemptions have on the total output of TDS. While other methods stayed constant for most of the time, large variability was seen in CWT, with discharge exemptions, contributing to the overall change seen in TDS loads. Figure used with permission from Cambridge Press and Dr Jessica Wilson (Wilson & VanBriesen 2012).

structural integrity) ranged from 3 to 43 % of wells surveyed around the globe. Although sustained casing pressure does not indicate environmental contamination has occurred, it does warrant a need for extensive research specific to each location where HVHF operations take place. When combined with the risk of disposal pipeline rupture or bursting, an increase in traffic, and an increase in the rates of traffic accidents as seen in some areas (Ridlington & Rumpler 2013), it quickly becomes apparent that accidental discharges are of great concern to industry and the public.

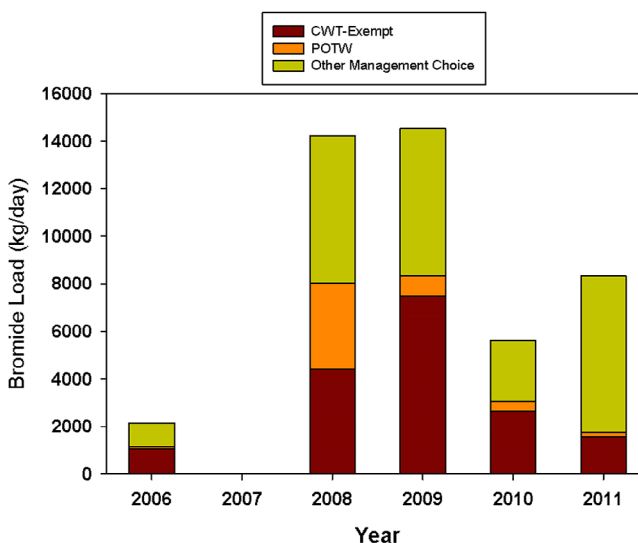


Figure 9. (Color online) Total Bromide load introduced into Pennsylvania surface waters by wastewater treatment plants during a five-year interval. This figure shows the trend of Bromide loads over five years and preferred treatment method used. Like in the case of TDS, overall deposition is highly influenced by exempt CWT treatment methods. Figure used with permission from Cambridge Press and Dr Jessica Wilson (Wilson et al. 2012).

Due to the high amounts of liquid used, the nature of its contents, and proximity to bodies of surface water, accidental discharges can have drastic effects on communities and the ecosystem. Furthermore, because some areas, such as Alberta, Canada, require companies to self-report spills, and well failure incidents, data on contamination frequency and extent are highly dependent on cooperation from the companies involved. To demonstrate the variation of well failure, we look at results of a study by Erno and Schmitz (1996), which found that of 1230 oil and gas wells near Lloydminster Canada, 23 % exhibited surface and soil gas leakage. However, regulatory data from the US EPA, cites rates of sustained casing pressure, near Pavillion, Wyoming, to be at 1 to 10 %, while groundwater contamination rates reach a maximum of 0.1 % of wells (DiGiulio et al. 2011). While keeping in mind the sheer number of wells located in the USA alone, 0.1 % is still a significant amount of contamination seen in just the groundwater matrix. Additionally, studies in Pennsylvania found over 100 spill and leak violations in a period of 6 years starting in 2008 (Vengosh et al. 2014), while Weld County, Colorado, documented 77 surface spills, that eventually impacted groundwater quality, in a one-year period beginning in July 2010 (Gross et al. 2013).

Recent events have raised concerns about the dangers of HVHF operations in areas like North Dakota and Montana, where HVHF operations are rapidly expanding (Hiriji 2015). As reported by Zahara Hirji (2015), on 17 January 2015, an oil pipeline, transporting crude oil from HVHF unconventional wells, and conventional oil drilling sites, leaked and released 40,000 gallons into the Yellowstone River in Montana. The nearby town of Glendive was cautioned not to consume the water and not to use it for cooking. Wintery conditions proved a challenge to clean-up crews, with ice keeping them from properly remediating the spill, allowing oil/chemicals to spread down the river.

The Yellowstone River experienced another pipeline incident in 2011, when the Exxon Silvertip Pipeline released 63,000 gallons of oil into the river. This event occurred just outside of the Yellowstone National Park and caused the evacuation of residents along a 20-mile stretch of the river (Associated Press 2011). A prompt response was needed to minimize risk and to prevent the harmful plume from

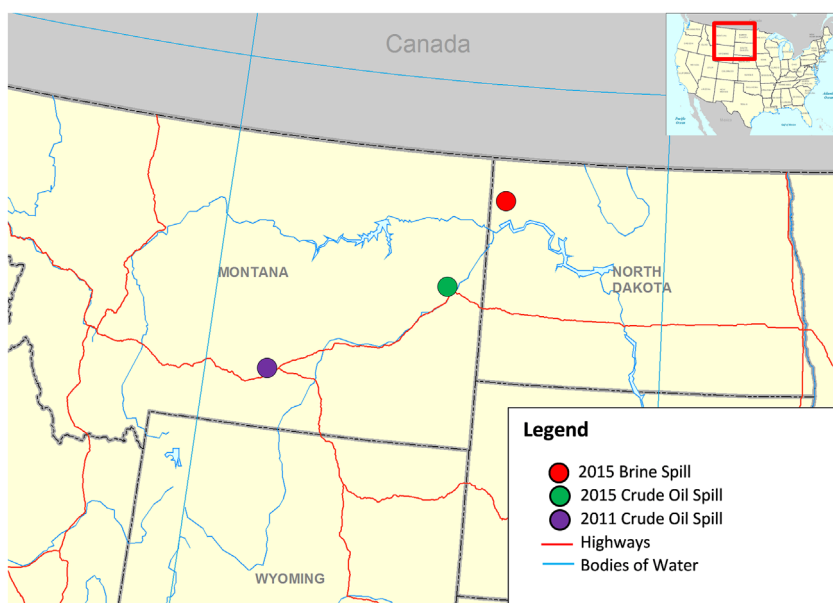


Figure 10. (Color online) Approximate locations of HVHF operation related spills occurring in 2011 and 2015. Locations approximated using reports by Associated Press (2011), MacPherson & Flesher (2015), and Hiriji (2015). The crude oil spill which occurred in 2011, spilled a total of 63,000 gallons of crude oil into the Yellowstone River, a smaller amount than the 2015 spill (40,000 gallons). The North Dakota brine spill was much larger in magnitude with three million gallons of brine being released. Pipeline leaks were the common factor across all three incidents. Map was generated using Esri ArcMap10 software (Esri 2014).

spreading down the Yellowstone River, which ultimately empties into the Missouri River, a source of drinking water for millions (Associated Press 2011).

A similar incident occurred in North Dakota where three million gallons of brine (resulting from HVHF operations) was released into nearby creeks due to a pipeline leak (MacPherson & Flesher 2015). The spill occurred on 6 January, 2015, affecting two creeks (Blacktail and Little Muddy Creek) in the area (MacPherson & Flesher 2015). They also reported that, as a result of the spill, Blacktail Creek will be fully drained and continuously monitored, while Little Muddy Creek will not be drained, due to its larger size, but will have to undergo a major cleanup process (2015). The environmental impact from the incident has not been determined and drinking water has not been affected (MacPherson & Flesher 2015). Figure 10 shows the approximate location of the three spills as discussed above and reported by news sources.

These accidental discharges are relatively recent events and the consequences to wildlife and human health will not be known for some time; however, Kassotis et al. (2013) has published an article in *Endocrinology* dealing with containment failures during HVHF operations and the presence of endocrine disrupting chemicals (EDCs) at spill sites. This study examined sites in a heavily fracked area, Garfield County, CO. Each individual site had from 43 to 136 natural gas wells within one mile of the location that had experienced a contamination of some type within six years of the study. The study focused on sampling each location and monitoring for presence of known, as well as suspected, EDCs. Several chemicals were found to have endocrine disrupting activities and the presence of these chemicals was linked to HVHF well pads. The study linked spills, much like the ones discussed above, with potentially harmful effects to endocrine systems of humans and wildlife.

Surface water risks attributed to HVHF operations

Increasing cancer risk

Hydraulic fracturing has been associated with dangerous and potentially carcinogenic compounds, such as benzene, xylene, and naturally occurring radioactive materials (NORM) (IRIS 2000, 2014; US EPA 2015b). As deep drilling operations are conducted, a large amount of potentially dangerous constituents in underground soil are liberated. These soil constituents can find their way to the surface during extraction of natural gas and oil, and during the removal and collection of flowback and produced waters. As these naturally occurring constituents tend to be radioactive (e.g. radium isotopes), there is a risk that untreated brines, waters, and sludge may contribute to cancer risk due to increased exposure to NORM (US EPA 2012a, 2015b). Although the frequency of exposure is not well documented, the risk of coming in contact with NORM associated with HVHF fluid waste is potentially highest among employees, who may come in contact with untreated byproducts of the HVHF process during pumping into storage ponds, or spills during transport. If containment of sludge and byproducts fails, NORMs may also find their way to surface waters and pose risks to recreational water users and wildlife.

Concerns involving NORM compounds include their introduction into drinking water supplies. By contaminating surface waters via accidental spills, infrastructure failure, and containment failures, a pathway is established for public exposure to these radioactive compounds. Although drinking water plants address the issue of NORM in water supplies, exposure in recreational water is still a valid concern (US EPA 2015b). Accidental ingestion, inhalation, and in some cases dermal contact with NORM's (e.g. radium isotopes) in contaminated water, can have both carcinogenic and DNA altering effects (US EPA 2012a). It should be noted that NORM constituents of soil can also pose a threat to the public via illegal dumping of sludge, brine, and untreated water (Cooley and Donnelly 2014).

Treatment of polluted source water may also prove problematic; stressing treatment plants beyond effectiveness may become a reality due to the many different contaminants and variables that must be accounted for in a heavily fracked area. As discussed above, factors introduced by HVHF activities include potential spills of chemicals, sludge, and brine spills (Hiriji 2015; MacPherson & Flesher

2015). These spills strain treatment facilities to a point where improper treatment of water can occur, endangering all residents of the area.

An example of an over-stressed treatment plant occurred on January 22, 2014, in West Virginia, when a leaking storage tank holding crude MCHM (4-(Methoxymethyl) cyclohexane methanol), contaminated a river that served as source water for Charleston (Cooper 2014). A plume was taken up by an intake pipe, where it saturated the activated charcoal filters. Unable to further filter the chemicals, the finished water was laced with contaminants. The city had to abstain from using faucet water and address the issue (Cooper 2014).

HVHF processes may also contribute to overall cancer risk via air pollution. During the lifetime of a typical well, large quantities of hydrocarbons are emitted (McKenzie et al. 2012). Some of these hydrocarbons, such as benzene and xylene, are known carcinogens, while other chemicals are irritants, causing lung irritation, light headedness, headaches, and other conditions (US EPA 2013a).

During the study by McKenzie et al. (2012), over 163 air samples were gathered for analysis. Four HVHF well pads were sampled from four separate companies in four cardinal directions (N, S, E, and W). Furthermore, four samples were taken at varying distances from each well, ranging from 40 to 153 m. To ensure consistency and minimize confounding variables, samples were taken upwind of traffic and agricultural areas and were analyzed by US EPA approved labs using US EPA's standard methods (2012). These samples were then used to calculate the risk of developing adverse health outcomes for 20- and 340-month periods.

The study found that benzene, ethylbenzene, o-xylene, and toluene were the chemicals that contributed more to a risk of developing adverse health outcomes. Calculations then showed that cancer risk, in areas greater than 1/2 mile from the well pad, was significantly lower than areas within the 1/2 mile zone. Furthermore, samples collected during the well completion phase were found to have more benzene, ethylbenzene, toluene and o-xylene than samples collected during gas collection. These samples contained 2.7, 4.5, 4.3, and 9.0 times more, respectively, of each chemical during well completion than during gas collection (McKenzie et al. 2012). The risk of developing cancer from exposure to these chemicals increases as distance from the drill site decreases.

The above study concluded that chronic exposure to HVHF-related air pollution contributed to an overall cancer risk, especially within 0.5 miles of the HVHF well (McKenzie et al. 2012). These main risk factors are a product of benzene, ethylbenzene, o-xylene, and toluene. Further studies are needed to assess occupational hazards and hazards to public health. (McKenzie et al. 2012).

Increased turbidity in water: impacts on drinking water quality and health

The hydraulic fracturing process is designed to be as efficient and high yielding, as possible. Consequently, areas with accessible shale resources can become dense with HVHF wells. An example is Washington County in Pennsylvania, holding 1100 wells alone during 2011 (Amico et al. 2011). Areas being with HVHF wells are usually in rural, undeveloped, remote areas which lack infrastructure and require land clearing and impromptu infrastructure construction. Paved roads are costly, and HVHF wells are ultimately temporary structures; therefore, dirt roads, unpaved parking lots, and unpaved well lots are common. These conditions result in several concerns involving the HVHF process, as outlined below.

When construction of a well begins, large areas of vegetation are removed in order to construct the well pad, containment pits and pods, roads, and parking to accommodate the thousands of truck trips that are required to operate a well. As previously mentioned, up to eight hectares of land may need to be cleared to ensure proper operation, a number that varies according to well location and characteristics (Johnson 2010; Entrekin et al. 2011). With decreased amounts of vegetation in an area, combined with heavy winds, rains, and increased truck traffic (each pad may see 3950 truck trips during early development) (NYSDEC 2011), increased amounts of sediment are transported to nearby bodies of water (Williams et al. 2007). In fact, Williams et al. (2007) show that one site was

contributing sediment at an estimated rate of 54 tonnes per hectare, per year, numbers that are comparable to small construction sites.

According to a 2007 US EPA report, a 0.404 hectare construction site with no existing runoff controls present can contribute the same amount of sediment as a 6.464 acre meadow. This estimate works out to be a runoff of 30–40 metric tons of sediment each year. Although, the US EPA has regulations requiring use of runoff controls on construction sites, and permit systems are in place, we must consider that hydraulic fracturing is expanding worldwide and that certain regions will not have the same stringent regulations in place. We also must realize that dirt roads are constructed leading to well pads, and that the increased traffic (discussed earlier) around well pads can disperse eroded soil particulates into the air, and beyond runoff boundaries.

The increased amount of sediment and eroded soil produced by well operations can contribute to turbidity increases in nearby bodies of water (Williams et al. 2007), which has been known to cause issues with drinking water treatment because it increases the survival rate of some pathogens, specifically *Giardia* and *Cryptosporidium* (LeChevallier and Norton 1992). As turbidity increases, disinfection procedure must be adjusted to compensate for potentially ineffective standard techniques (US EPA 1999). Turbidity monitoring is essential to the water treatment process as it dictates the amount of initial disinfectant needed for the process. Turbidity also decreases the effectiveness of UV disinfection, making initial treatment of water even more difficult (US EPA 1999). This occurs because soil particles in the turbid water, if not properly removed, can shield microbes from UV light (US EPA 1999). By decreasing the effectiveness of UV radiation as a primary disinfectant, increases in turbidity can dictate an increase in the use of chemicals for water treatment. This could lead to higher costs, more chemical demand, and an increase in the formation of DBPs, that are mutagenic and linked to cancer development in animals and humans (WHO 2005). Although regulations on runoff controls and sediment discharge exist in the USA, the same cannot necessarily be said for other countries. Additionally, failure of runoff controls cannot be dismissed and should be accounted for when considering surface water integrity.

Recreational water use must also be taken into account. As stated above, an increase in turbidity increases the pathogen survival time, specifically *Giardia*, *Cryptosporidium*, and many other pathogens (LeChevallier & Norton 1992). Because an increase in turbidity shields these organisms from UV damage, one can infer that recreational use of an affected lake could result in increased exposure to these pathogens, especially after extreme precipitation, windy days, or other events leading to large amounts of sediment deposition. With the projected long-term use of HVHF a likely reality, extreme weather events attributed to climate change may play an important role in an increased introduction of sediment, due to runoff into bodies of water near HVHF sites (Emori & Brown 2005; US EPA 2014a).

Conclusion

In summary, HVHF presents many potential risks to multiple environmental matrices, especially surface waters. Unconventional wells in Pennsylvania have been found to have a higher risk of casing and cement failure, in contrast to conventional wells in the same locations. Treatment of produced water in Pennsylvania in 2008/2009 also contributed to significantly increased total dissolved salt and bromide loads in drinking water sources throughout Pennsylvania. Levels of dissolved salts and bromide decreased in 2010, but were still higher than those found in 2006.

HVHF operations pose a potential risk to human health, both directly and indirectly, by introducing dangerous contaminants into water supplies. Recreational use of these waters or improper treatment of drinking water, may pose dangerous health outcomes. An increased cancer risk may be associated with HVHF activity, as it opens up new pathways for exposure to NORM and carcinogenic compounds. Contact with HVHF chemicals, or their products, can cause harm to the endocrine system with negative outcomes to the sexual organs. Construction and use of HVHF infrastructure also considerably contributes to erosion in surrounding areas, potentially impacting water treatment and increasing pathogen survival time in surface waters.

Future research and resources should focus on collecting surface water quality data before, and after, HVHF operations. In this manner, changes that are observed can be more closely associated with HVHF operation. This can be accomplished by minimizing the confounding factors and determining if the HVHF process is the only, or main, contributor of contamination. The data could then be used to construct predictive models for future HVHF sites, and help companies to more efficiently treat, and dispose of, produced water, while informing drinking water treatment plants to screen for potential contaminants, and suggesting methods for remediation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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A Framework for Identifying Organic Compounds of Concern in Hydraulic Fracturing Fluids Based on Their Mobility and Persistence in Groundwater

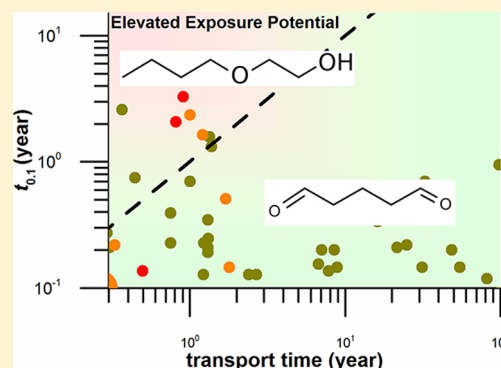
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S Supporting Information

ABSTRACT: We developed a screening framework for identifying organic components of hydraulic fracturing fluid with increased probability of exposure via groundwater based on mobility, persistence, toxicity, and frequency of use. Of 996 organic fracturing fluid compounds identified by the U.S. Environmental Protection Agency and FracFocus for four states, data were available to perform an initial screening of 659 compounds for sufficient mobility and persistence to reach a water well under fast and slow groundwater transport scenarios. For the fast transport scenario, 15 compounds identified on at least 50 FracFocus reports were predicted to have an elevated exposure potential, which was defined as $\geq 10\%$ of the initial concentration remaining at a transport distance of 94 m, the average setback distance in the United States. Of these 15 compounds, two were identified on $>20\%$ of FracFocus reports (naphthalene and 2-butoxyethanol), four were compounds identified on $>5\%$ of reports, and three had health-based standards.



INTRODUCTION

Significant technological advances in horizontal drilling and hydraulic fracturing are allowing extraction of hydrocarbons from low-permeability formations that were previously not developed because of limited recovery.¹ Rapid expansion of the use of these technologies has generated concern about degradation of water quality in aquifers.^{1–5} Fracturing fluids are pumped into the shale formation at high pressures to induce fractures and carry proppants to hold open the fractures and create a flow path for the hydrocarbons to the well. A typical fracturing fluid composition includes $\sim 90\%$ water, $\sim 9\%$ proppants, and $\sim 1\%$ chemical additives, including surfactants, friction reducers, gelling agents, and biocides.^{6,7} Each additive is a mixture of compounds, the types and concentrations of which vary substantially on the basis of basin characteristics as well as operator specifications.⁶

Subsurface pathways that may result in the release of fracturing fluids to aquifers include failure of well integrity, improperly abandoned wells, and existing faults or fractures in geologic formations between the target formations and aquifers.^{1–3,6} The probability of groundwater contamination by these pathways is generally thought to be low.⁸ Surface pathways include spills from the transport, storage, and handling of fracturing fluid additives as well as flowback water from the formation.^{2,3,9} Surface spills and releases are the more likely groundwater contamination pathway.^{2,5,8,10}

Human exposure to a harmful fracturing fluid constituent requires transport to a potential receptor,⁵ and the broad variety of compounds possibly used in fracturing fluids poses a challenge when evaluating transport potential.⁶ Previous studies of potential drinking water contamination by fracturing fluids have focused on characterizing the toxicity of the individual compounds,^{11–14} with consideration of environmental exposure potential for only a limited number of compounds^{9,13,14} and not mixtures.¹⁵ The variety of compounds hinders baseline groundwater monitoring due to analytical limitations of a comprehensive analysis of possible constituents.¹⁶ Effective groundwater monitoring should focus on constituents most likely to be present.¹⁷

Our objectives are (1) to develop a screening framework for identifying fracturing fluid compounds that are sufficiently mobile and persistent to be transported through aquifers by comparing the time for a compound to degrade to a predicted groundwater transport time and (2) to perform an initial screening using available data for 659 compounds. Degradation products and mixture interactions were not considered.

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Table 1. Frequently Used Organic Compounds in Hydraulic Fracturing Fluids and Corresponding Screening Framework Parameters^a

compound	additive purpose ^b	FracFocus frequency (%) ^c	toxicity cat. ^d	K_d (L kg _{sed} ⁻¹) ^e	$t_{1/2}$ (d)	ref ^f
methanol	corrosion inhibitor, surfactant, nonemulsifier, scale inhibitor, biocide, cross-linker	76.5	HAI	1×10^{-3}	1	25
hydrotreated light petroleum distillates	friction reducer, gelling agent, cross-linker	70.0	no HAI	2×10^2	231	28
2-propanol	corrosion inhibitor, nonemulsifier, surfactant	50.1	no HAI	3×10^{-3}	14	26
ethylene glycol	cross-linker, scale inhibitor, corrosion inhibitor, friction reducer	49.7	HAI	2×10^{-4}	24	25
guar gum	gelling agent	45.2	no HAI	3×10^{-1}	0.1	29
ethanol	surfactant, biocide	34.2	no HAI	2×10^{-3}	2	27
glutaraldehyde	biocide	33.3	no HAI	2×10^{-3}	0.3	27
propargyl alcohol	corrosion inhibitor	32.7	HAI	2×10^{-3}	13	27
acetic acid	buffer, iron control	31.7	no HAI	7×10^{-6}	7	28
citric acid	iron control	23.4	no HAI	3×10^{-9}	0.4	28
heavy aromatic petroleum naphtha	surfactant, nonemulsifier, inhibitor, corrosion inhibitor	23.3	no HAI	7×10^{-1}	6	21
2-butoxyethanol	surfactant, corrosion inhibitor, nonemulsifier	22.8	HAI	8×10^{-3}	56	26
naphthalene	surfactant, nonemulsifier, corrosion inhibitor	22.0	HAI	7×10^{-1}	258	25
sorbitan monooleate	friction reducer, biocide, corrosion inhibitor	20.7	no HAI	2×10^{-3}	20	28

^aCompounds were designated frequently used if they were identified on >20% of FracFocus disclosure reports. ^bFunction of additive in which each compound was identified as an ingredient, reported by the EPA.⁵³ FracFocus does not report the specific purpose of each compound within an additive, and a compound may be used as an ingredient in multiple additives.⁵³ ^cPercentage of FracFocus reports identifying use of compound out of 50024 reports acquired for the states of Colorado, North Dakota, Pennsylvania, and Texas. ^dIndicates availability of health assessment information (HAI). No frequently used compounds had health-based standards (MCLs). ^eCalculated according to eq 3. ^fReferences for degradation kinetics data.

METHODS

Compound Sources and Selection. A total of 996 unique organic compounds were identified. Of these, 641 were identified from unique Chemical Abstract Service (CAS) numbers reported on FracFocus Chemical Disclosure Registry reports¹⁸ acquired for Colorado (7772 reports), North Dakota (5662), Pennsylvania (4312), and Texas (32278) as of November 2014. By 2012, all four states required disclosure on FracFocus.¹⁹ Additional compounds were identified from a list compiled by the Environmental Protection Agency (EPA).²⁰ Some compounds were identified on both lists (331); however, 355 were identified only by the EPA. Compounds were not consistently reported on FracFocus until 2011,¹⁸ while the EPA list includes compounds disclosed between 2005 and 2010. Consequently, there are compounds considered in this analysis that operators may no longer use but are of interest with respect to potential legacy contamination. Compounds that are considered proprietary may not be identified by CAS number on the FracFocus reports.¹⁹ Finally, both the EPA list and FracFocus include compounds with no apparent use in fracturing fluids, for instance, due to errors in the reported CAS number. To account for compounds with no indication of current or apparent use in fracturing fluids, compounds were only designated as having an elevated exposure potential if they were predicted to have $\geq 10\%$ of the initial concentration remaining at the setback distance and were identified on ≥ 50 FracFocus reports (0.1% of reports acquired).

Of the 996 compounds, CAS numbers or unique structures could not be identified for 337 compounds (Methods of the Supporting Information); these were classified as “no data” and were not screened, which left 659 organic compounds to be screened. Degradation products of the screened compounds were not considered.

Screening Framework. Compounds were screened by comparing the time to degrade to 10% of the initial

concentration ($t_{0.1}$) against the transport time to a distance of 94 m (t_{94}), the national average required setback distance.¹⁹ The average setback distance represents a horizontal transport distance for a contaminant from an oil and gas well to a domestic well. The framework simulates horizontal transport in an aquifer from a point of release (e.g., a failed well casing, a spill that has migrated through the vadose zone) to a drinking water well. Two transport scenarios were considered: a highly porous aquifer with relatively fast groundwater velocity and a less porous aquifer with slower velocity. Compounds identified on ≥ 50 FracFocus reports and predicted to have $\geq 10\%$ of the initial concentration remaining at the transport distance were predicted to have an elevated exposure probability.

Mobility. The transport time of each compound, t_{94} , was calculated using an average linear groundwater velocity, v_w , as

$$t_{94} = \frac{d_c R}{v_w} \quad (1)$$

where d_c is the setback distance (94 m) and R is the retardation coefficient, calculated as

$$R = 1 + \rho_s \left(\frac{1}{n} - 1 \right) K_d \quad (2)$$

where ρ_s is the density of the aquifer sediment (assumed to be 2.65 kg L⁻¹), n is the porosity, and K_d is the distribution coefficient (liters per kilogram of sediment). The distribution coefficient was estimated to include sorption of the neutral form of the compound to organic matter and sorption of the cationic form of organic bases by ion exchange:

$$K_d = \alpha_n K_{oc} f_{oc} + \alpha_c K_{d,ex} \quad (3)$$

where α_n is the neutral fraction of the organic compound, α_c is the cationic fraction of organic bases, K_{oc} (liters per kilogram of organic carbon) is the organic carbon partition coefficient, $K_{d,ex}$ (liters per kilogram of sediment) is the ion exchange coefficient, and f_{oc} is the fraction of organic carbon of the sediment

(assumed to be $0.001 \text{ kg}_{\text{oc}} \text{ kg}_{\text{sed}}^{-1}$). The K_{oc} of each compound was estimated by the EPA's widely used Estimation Program Interface (EPI) Suite program.²¹ We estimated $K_{\text{d,ex}}$ for 83 bases that were $\geq 10\%$ ionized at pH 7 assuming a low cation exchange capacity consistent with an alluvial sediment allowing fast transport (Methods of the Supporting Information). Ion exchange was considered negligible for ionized organic acids because the anion exchange capacity of typical alluvial aquifer sediments is relatively low.^{22,23}

The transport time was calculated for scenarios of fast (v_w of 1 m day^{-1}) and slow (v_w of 0.01 m day^{-1}) groundwater flow. The fast transport scenario is consistent with velocities reported for alluvial deposits.²⁴

Persistence. Persistence was evaluated using a "tenth-life", $t_{0.1}$, which was calculated from a compound's half-life ($t_{1/2}$) as

$$t_{0.1} = t_{1/2} \left[\frac{\ln(0.1)}{\ln(0.5)} \right] \quad (4)$$

For 312 compounds, biodegradation and hydrolysis half-lives were determined from published experimental data.^{21,25–35} When available, degradation kinetics in groundwater attenuation studies or anaerobic environments were chosen over aerobic kinetics to better represent likely conditions in a groundwater contaminant plume. For the other 347 compounds, experimental degradation data were not available. These compounds were screened using an estimated time required for complete primary biodegradation in an aerobic aquatic environment predicted by BIOWIN 4, which calculated a rating corresponding to an expected degradation time range (Table S2 of the Supporting Information).^{21,36} Half-lives assigned to the BIOWIN-predicted time range intervals³⁷ were used to adjust the predictions to an estimated $t_{0.1}$ (Methods of the Supporting Information).

Toxicity. Compounds were evaluated for potential toxicity using National Primary Drinking Water Regulations (NPDWR)³⁸ and health assessment information, including oral reference doses, inhalation reference concentrations, and carcinogenic risk slope factors published by the EPA Integrated Risk Information System (IRIS).³⁹ Three categories were defined: 14 compounds had a maximum contaminant level (MCL) that could be enforced under NPDWR, 53 compounds had published health assessment information (e.g., reference doses) but no MCL, and 592 compounds had no MCL and no health assessment information.

Frequency of Use. The frequency of use was assessed by counting the number of FracFocus reports on which a compound's CAS number appeared. Table 1 summarizes the screening parameters for 14 frequently used compounds identified on $>20\%$ of FracFocus reports. Frequency of use was assessed on a national scale and could misrepresent the prevalence of a compound regionally.

RESULTS AND DISCUSSION

Compounds with Elevated Exposure Potential. For the fast transport scenario, 41 compounds screened using published degradation data were predicted to have $\geq 10\%$ of the initial concentration remaining at the transport distance (Figure 1). Of these, 15 compounds were also identified on ≥ 50 FracFocus reports and were predicted to have an elevated exposure potential (Table 2). Because some compounds excluded or rarely identified on FracFocus could be relevant for assessing potential legacy contamination, all compounds predicted to be

sufficiently mobile and persistent were identified (Table S1 of the Supporting Information); however, they were considered to have an elevated exposure potential only if they were identified on ≥ 50 reports.

Three of the 15 compounds with elevated exposure potential have health-based standards (MCLs): acrylamide and the petroleum hydrocarbons ethylbenzene and xylenes. The occurrence of these compounds at concentrations above their MCLs would necessitate regulatory action. Acrylamide was identified on 3.2% of FracFocus reports as a residual ingredient in nonhazardous acrylamide polymers commonly used in fracturing fluids as friction reducers.^{13,40} The petroleum hydrocarbons were rarely identified as individual additives on FracFocus (e.g., ethylbenzene, 0.30% of reports), but they are minor constituents of some petroleum-based additives like hydrotreated light petroleum distillates [$<0.5\%$ (w/v)],^{41,42} identified as an ingredient in friction reducers on 70.0% of reports. Benzene, also a minor constituent of petroleum distillates, was predicted to be sufficiently mobile and persistent by the screening framework but was individually identified on <50 reports.

Six of the 15 compounds with elevated exposure potential have health assessment information (e.g., reference doses), but no MCLs. The occurrence of these compounds at concentrations above their reference doses could be a concern for human health but would not necessitate regulatory action. Two of six were frequently used: 2-butoxyethanol (22.8% of FracFocus reports) and naphthalene (22.0%). Both compounds are commonly identified as ingredients in surfactants, corrosion inhibitors, and nonemulsifiers, and naphthalene is a constituent of some petroleum-based additives.^{41,43} A recent study detected trace concentrations of 2-butoxyethanol in addition to unresolved complex mixtures of organic compounds in a domestic water well, concluding that drilling or fracturing fluids used in nearby gas wells likely caused the observed groundwater contamination.⁴⁴ *N,N*-Dimethylformamide, an ingredient in corrosion inhibitors, was identified on 9.1% of FracFocus reports. Three of the six compounds were rarely identified ($<5\%$ of reports).

The remaining six of the 15 compounds with elevated exposure potential did not have health assessment information; these compounds are currently not assessed in the EPA IRIS database. Three of the six were more commonly used in fracturing fluids [polysorbate 80 (12.6% of FracFocus reports), 2-mercaptoethanol (8.7%), and 2-ethylhexanol (7.2%)], and the other three compounds were rarely identified.

For the fast transport scenario, six of the compounds screened using the BIOWIN-estimated biodegradation time ranges were predicted to have $\geq 10\%$ of the initial concentration remaining at the transport distance, and four of the six were predicted to have elevated exposure potentials but were rarely identified on FracFocus (Table 2 and Figure S2 of the Supporting Information). In the slow transport scenario, no compounds were predicted to have an elevated exposure potential because degradation to $<10\%$ of the initial concentration occurred by the time the compounds reached the transport distance (Figures S1 and S2 of the Supporting Information).

Frequently Used Compounds. Frequently used compounds identified on $>20\%$ of FracFocus reports are labeled in Figure 1. Ethylene glycol, sorbitan monooleate, 2-propanol, and propargyl alcohol were plotted just below the region designating elevated exposure potential. Both ethylene glycol

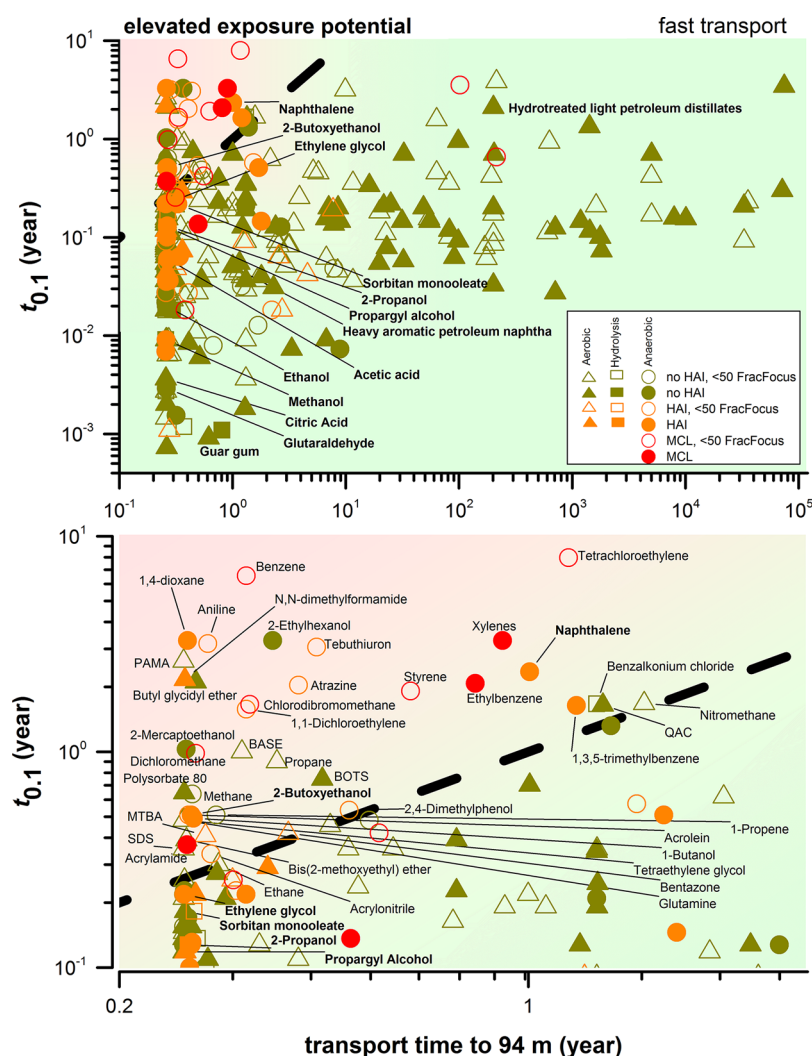


Figure 1. Screening framework plot of time to 10% of initial concentration ($t_{0.1}$) vs. transport time to 94 m (the average setback distance in the United States) for 312 compounds with degradation data available. (top) The elevated exposure potential zone ($\geq 10\%$ of the initial concentration predicted to remain at the setback distance) is defined as the area above the 1:1 line; (bottom) the elevated exposure potential zone is shown in detail. The transport time is calculated for the fast groundwater transport scenario. The 41 compounds predicted to have $\geq 10\%$ of their initial concentration remaining at the transport distance are labeled in the bottom plot (compound abbreviations are defined in Table 2 and Table S1 of the Supporting Information). Frequently used compounds (appearing on $>20\%$ of FracFocus reports) are indicated by bold labels. Toxicity is represented using three categories: compounds with maximum contaminant levels (MCL, red symbols), compounds with health assessment information but not MCLs (HAI, orange symbols), and compounds without MCLs and HAI (no HAI, yellow symbols). Empty symbols represent data for compounds appearing on <50 FracFocus reports and filled symbols those appearing on ≥ 50 reports. The symbol shape indicates whether the half-life used to calculate the $t_{0.1}$ value represents anaerobic biodegradation (circles), aerobic biodegradation (triangles), or abiotic hydrolysis (squares).

and propargyl alcohol have oral reference doses, and while ethylene glycol's dose is relatively high, occurrence at concentrations above the reference dose could be a health concern.

Frequently used compounds of elevated exposure potential could be utilized as indicators of contamination by fracturing fluids. Groundwater monitoring programs and studies evaluating potential migration of fracturing fluids have focused on conservative ions such as chloride and bromide;^{17,45,46} however, the source of these ions is not readily distinguished.^{45,46} The screening framework may be applied to prioritize future research needs, including measuring fate and transport parameters for frequently used mobile and persistent compounds relevant to human health risk assessments or that

could be considered for groundwater monitoring programs to help indicate fracturing fluid migration.

Uncertainty and Limitations. The persistence assessment is highly uncertain because degradation data are limited for many fracturing fluid compounds² and kinetics can vary depending on site conditions.^{32,47} Most of the biodegradation half-lives used to calculate $t_{0.1}$ were measured or estimated in oxic environments, and kinetics under anoxic conditions common to groundwater contaminant plumes are generally slower than aerobic rates.^{47,48} The half-life assumed in the screening framework allows for the comparison of a broad variety of compounds; however, degradation rates used will not be applicable for all site conditions. The lack of published degradation data required that 53% of compounds were screened using estimates of aerobic biodegradation time ranges.

Table 2. Compounds Predicted To Have an Elevated Exposure Potential Because They Were Identified on ≥ 50 FracFocus Reports (0.1% of reports acquired) and Predicted To Have $\geq 10\%$ of the Initial Concentration Remaining at the 94 m Transport Distance (the average setback distance in the United States)

compound	additive purpose ^a	FracFocus frequency (%) ^b	toxicity cat. ^c	$t_{0.1}$ (year) ^d	t_{94} (year) ^e
acrylamide	friction reducer	3.2	MCL	0.37 (an)	0.26
ethylbenzene	corrosion inhibitor	0.30	MCL	2.1 (an)	0.81
xylene ^f	corrosion inhibitor, surfactant, solvent	2.3	MCL	3.3 (an)	0.90
1,3,5-trimethylbenzene	surfactant	0.75	HAI	1.6 (an)	1.2
1,4-dioxane	surfactant	1.2	HAI	3.3 (an)	0.26
1-butanol	surfactant	2.1	HAI	0.49 (an)	0.27
2-butoxyethanol	surfactant, corrosion inhibitor, nonemulsifier	22.8	HAI	0.51 (an)	0.27
<i>N,N</i> -dimethylformamide	corrosion inhibitor	9.1	HAI	2.2 (ae)	0.26
naphthalene	surfactant, nonemulsifier, corrosion inhibitor	22.0	HAI	2.4 (an)	1.0
2-ethylhexanol	nonemulsifier, surfactant	7.2	no HAI	3.3 (an)	0.37
2-mercaptoethanol	iron control	8.7	no HAI	1.0 (an)	0.26
benzene, 1,1'-oxybis-, tetrapropylene derivatives, sulfonated, sodium salts (BOTS)	scale inhibitor, surfactant	1.3	no HAI	0.75 (ae)	0.44
butyl glycidyl ether	resin	0.93	no HAI	2.1 (ae)	0.27
polysorbate 80	surfactant	12.6	no HAI	0.65 (ae)	0.26
quaternary ammonium compounds, dicoco alkylmethyl, chlorides (QAC)	corrosion inhibitor, biocide	4.7	no HAI	1.7 (ae)	1.3
bis(hexamethylenetriamine penta methylene phosphonic acid) (BMPA)	scale inhibitor	1.2	no HAI	0.55 (ae) ^g	0.26
diethylenetriaminepenta (methylene-phosphonic acid) (DMPA)	scale inhibitor	0.16	no HAI	0.55 (ae) ^g	0.26
FD&C blue no. 1	gelling agent	0.39	no HAI	0.34 (ae) ^g	0.26
tetrakis(triethanolamino) zirconium(IV) (TTZ)	cross-linker	2.8	no HAI	0.34 (ae) ^g	0.26

^aFunction of additive in which each compound was identified as an ingredient, reported by the EPA.⁵³ FracFocus does not report the specific purpose of each compound within an additive, and a compound may be used as an ingredient in multiple additives.⁵³ ^bPercentage of FracFocus reports identifying the use of a compound out of 50024 reports acquired for the states of Colorado, North Dakota, Pennsylvania, and Texas. ^cIndicates whether a compound has health-based standards (MCL), no MCL but health assessment information available (HAI), or no MCL and no health assessment information (no HAI). ^dCalculated according to eq 4. The conditions for which the compound's half-life was reported are noted for anaerobic biodegradation (an), aerobic biodegradation (ae), or abiotic hydrolysis (hy). ^eEstimated according to eq 1 for fast transport scenario. ^f"Xylenes" refers to the mixture of three isomers (ortho, meta, and para). Toxicity studies performed for the mixture.³⁹ Fate and transport parameters were determined for *m*-xylene because it is the dominant isomer in the mixture (45–70%).²⁷ ^gEstimated $t_{0.1}$ adjusted from the expected time range required for complete aerobic biodegradation predicted by BIOWIN 4.²¹

While models such as BIOWIN 4 are useful as an initial screening tool,^{36,49} the uncertainty of these predictions and the difficulty of extrapolating rates from one condition to another⁴⁷ illustrate the need for data on the degradation of many compounds used in fracturing fluids under conditions relevant for groundwater transport.

We did not consider possible interactions between co-contaminants, which may include solubility enhancement by cosolvents, mobility enhancement by association with surfactants,⁵⁰ or biodegradation inhibition by biocides.^{13,29} We also did not account for any chemical transformations that compounds may undergo downhole, or the formation of additional organic compounds in produced water.^{51,52} The degradation of some fracturing fluid compounds may yield intermediate products that are more hazardous than the parent compound,^{14,48} but the behavior of these degradation products was not considered.

Uncertainties in framework parameters, including the dependency of sorption on site-specific conditions and the variability of degradation rates, could push compounds above or below the 10% remaining threshold. Limitations such as neglecting possible mixture interactions, omitting degradation products and any intermediates formed downhole, and the exclusion of fate and transport parameters relevant to specific pathways (e.g., volatilization and dispersion) could also

influence the exposure potential. The 10% remaining threshold will not be appropriate for all scenarios because some compounds are hazardous at trace quantities and groundwater concentrations could vary considerably depending on the mass released and transport pathway. The toxicity assessment is limited because mixture toxicity was not considered and some hazardous compounds may not be included in the EPA's IRIS database. While it remains challenging to predict the exposure potential of fracturing fluid compounds in groundwater, the initial screening results prioritize compounds with elevated potential for human exposure based on our current knowledge. More data on the fate and transport of fracturing fluid organic constituents and their transformation products are needed to improve our understanding of the exposure potential in groundwater.

■ ASSOCIATED CONTENT

§ Supporting Information

Additional details about the methods and results (all compounds predicted to have $\geq 10\%$ remaining, slow scenario, predicted biodegradation time frames), screening parameters, and compounds classified as "no data". The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.5b00090.

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Notes

The authors declare no competing financial interest.

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Malignant human cell transformation of Marcellus Shale gas drilling flow back water[☆]



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ABSTRACT

The rapid development of high-volume horizontal hydraulic fracturing for mining natural gas from shale has posed potential impacts on human health and biodiversity. The produced flow back waters after hydraulic stimulation are known to carry high levels of saline and total dissolved solids. To understand the toxicity and potential carcinogenic effects of these wastewaters, flow back waters from five Marcellus hydraulic fracturing oil and gas wells were analyzed. The physicochemical nature of these samples was analyzed by inductively coupled plasma mass spectrometry and scanning electron microscopy/energy dispersive X-ray spectroscopy. A cytotoxicity study using colony formation as the endpoint was carried out to define the LC₅₀ values of test samples using human bronchial epithelial cells (BEAS-2B). The BEAS-2B cell transformation assay was employed to assess the carcinogenic potential of the samples. Barium and strontium were among the most abundant metals in these samples and the same metals were found to be elevated in BEAS-2B cells after long-term treatment. BEAS-2B cells treated for 6 weeks with flow back waters produced colony formation in soft agar that was concentration dependent. In addition, flow back water-transformed BEAS-2B cells show better migration capability when compared to control cells. This study provides information needed to assess the potential health impact of post-hydraulic fracturing flow back waters from Marcellus Shale natural gas mining.

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Introduction

Natural gas is believed to possibly be a bridge to transitioning from coal dependence. Currently natural gas fuels nearly 40% of the U.S. electricity generation, and the Marcellus Shale formation in the Appalachian Basin is on the forefront of gas-shale drilling for natural gas production in the United States (Pritz, 2010). Mining natural gas is not new, but the volume has soared in recent years because the new technique of high-volume horizontal hydraulic fracturing (HVHF). The concern surrounding the environmental, public health and social impacts of this method has increased accordingly. HVHF is an advanced technology that injects water, sand, and other ingredients at very high pressure vertically into a well about 6000 to 10,000 ft deep (Penningroth et al., 2013). The high pressure creates small fractures in the rock that extend out as far as 1000 ft away from the well. The pressure is reduced after

the fractures are created, which allows water from the well to return to the surface, also known as flow back water (Veil, 2010). The flow back water contains complex proprietary chemical mixtures, but also naturally occurring toxins such as metals, volatile organics, and radioactive compounds that are destabilized during gas extraction (Warner et al., 2012). On average, about 5.5 million gallons of water is used on average to hydraulically fracture each shale gas well, and 30% to 70% of the volume returns as flow back water (Veil, 2010). Currently discharge options of flow back water are: inject underground through an onsite or offsite disposal well; discharge to a nearby surface water body; transport to a municipal wastewater treatment plant or publicly owned treatment works; transport to a commercial industrial wastewater treatment facility; and/or reuse for a future hydraulic fracturing job either with or without some remediation (Pritz, 2010). Some commercial wastewater disposal facilities accept flow back and discharge the water after treatment under their own national pollutant discharge elimination system permits (Veil, 2010).

Metal pollution is a serious problem as they are taken up readily in the digestive tract and exhibit harmful effects on many tissues (Alomary et al., 2013; Rasmussen et al., 2013). Barium and strontium

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are abundant in the Marcellus Shale formation, and are easily dissolved and transported in wastewater after gas drilling activity (Pritz, 2010), which could potentially pose a threat to drinking water (EPA).

In contrast to the increased support of drilling and exploration by U.S. government agencies and rising concerns of impact on human and animal health within close proximity of the drilling sites (Bamberger and Oswald, 2012), knowledge of the health risks associated with the gas drilling wastewater is sparse. The question posed here is whether flow back water specific to the Marcellus Shale malignantly transforms cells, and if it does, what's the mechanism underlying tumorigenic potential of produced flow back water.

It has been challenging yet critical to choose a proper human cellular model to address this question. Immortalized human bronchial epithelial cells (BEAS-2B) have been widely used as a malignant cell transformation model to estimate the carcinogenesis capability of various environmental toxicants (Liao et al., 2007; Chang et al., 2010; Son et al., 2012; Yang et al., 2013). It has wild-type and functional p53 gene expression due to the loss of SV40 in passages after immortalization process, which provides a low spontaneous anchorage free growth, a quality of good cellular model for malignant cell transformation analysis (Lehman et al., 1993).

In this study we employed BEAS-2B as well established models (Lee et al., 1993; Chen et al., 2006; Sun et al., 2011; Passantino et al., 2013) to investigate the malignant cell transformation of Marcellus Shale gas drilling flow back water.

Material and methods

Cell culture and exposure

BEAS-2B (ATCC, Manassas, VA) cells were cultured as previously described (Sun et al., 2011; Passantino et al., 2013) at 37 °C in a humid 5% CO₂ atmosphere. BEAS-2B cells were seeded at 3×10^5 into 25 cm² polystyrene tissue culture flasks. The cells were treated with filtered (0.22 µm filter) produced flow back water collected from Bradford County, PA (a generous gift from Dr. Carl Kirby and Dr. Judy Zelickoff), and diluted with the appropriate medium to 0.13%, 0.25%, 0.5%, 1%, 2%, 4% or 8% (v/v). Control cells received distilled water or filtered water from a pristine lake located in Sterling Forest (SF), NY, diluted with the designated medium to 4% (v/v). The cells were cultured for various time intervals as indicated. Every 3 to 4 days, the cells were trypsinized, counted, and re-seeded into fresh 25 cm² flasks at a density of 3×10^5 viable cells per flask, and provided fresh media with the appropriate concentration of the flow back water sample.

Scanning electron microscopy (SEM)/energy dispersive X-ray spectroscopy (EDX)

The water samples were briefly sonicated in bath sonication for 1 min to make sure the solution was uniform. Both high density and low density samples were prepared. High density water samples were prepared with one drop of 100 µL of each sample dried on carbon tape in a class 100 clean room. For low density samples, 100 µL of each sample was spin coated onto a carbon tape surface pre-mounted to a SEM sample holder at 200 rpm before being air dried overnight in a class 100 clean room. Field Emission Scanning Electron Microscopy (FEI, The Netherlands) and Energy Dispersive Spectrometry (model Genesis 60S, by EDAX Company, USA) were performed to identify any particles in the sample and the chemical components of those particulates were analyzed by EDX.

Inductively coupled plasma mass spectrometry (ICP-MS)

A volume of 0.1 mL of each sample including flow back water and their filtrates was ionized in tubes with 1 mL HNO₃ (70%) at 140 °C for 5 h. Concentrations of the heavy metals were determined

by an ICP-MS (Perkin Elmer, Warsaw, Poland). BEAS-2B cells after 5 weeks of treatment with flow back water at 0.5% (v/v) were trypsinized and counted to determine the total cell number. The cell pellets with same amount of cells were then mixed with 3 mL of HNO₃ (70%) and incubated at 80 °C for 48 h, followed by cooling for 1 h to room temperature. After cooling, 3 mL of hydrogen peroxide (30%) was added to each tube, followed by incubation of the solution at 80 °C for 3 h. After suitable dilution of the digested materials with ultrapure water, levels of elements in the samples were determined by ICP-MS.

Colony formation and soft agar assay

Following treatment, BEAS-2B cells were trypsinized and counted using a hemocytometer to determine viability. Colony formation and soft agar assay were then conducted, and cells that exhibited anchorage free growth were collected for wound healing assay according to previously published procedure (Passantino et al., 2013); for detailed information, please see supplemental materials.

Cell migration assays

Matrigel (BD Biosciences, Bedford, MA) was reconstituted on the top surfaces of Transwell membranes at 100 µg protein/cm² of surface area. Transformed BEAS-2B cells (5×10^4 in 100 µL) were added to the upper chamber in serum free medium supplemented with 0.5 µM plasminogen. The bottom chamber contained DMEM supplemented with 10% FBS. The cells were allowed to invade for 24 h at 37 °C, at which time the Matrigel and cells that were associated with the top surfaces of the membranes were removed with cotton swabs. Cells that penetrated through the Matrigel to the underside surfaces of the membranes were fixed and stained with 0.1% Crystal Violet. Cells on the lower surface of the filter were enumerated using an ocular micrometer. Five fields were counted. Each experiment was performed twice with triplicate samples.

Wound healing assay

Cells (2×10^5) from each clone extracted from soft agar were plated into 35 mm culture dishes with a grid etched into the bottom. The cells were cultured in 1 × DMEM complete media until 100% confluent (4 days). The media were then replaced with 1 × PBS, and a single scratch was made across the monolayer using a 1 mL pipette tip held perpendicular to the plate bottom. The plate was washed twice

Table 1
ICP-MS analysis of filtered Marcellus Shale flow back.

Concentration (mg/L)	Well 1	Well 2	Well 3	Well 4	Well 5	SF
Al	0.301	0.044	1.268	1.102	1.057	N.D.
B	0.315	0.252	0.408	0.388	0.370	N.D.
Ba	452.4	474.6	596.5	606.8	701.6	N.D.
Bi	0.110	0.164	0.180	0.158	0.059	N.D.
Ca	470.7	974.9	707.8	608.9	502	1537
Co	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Fe	3.506	1.343	4.491	N.D.	N.D.	N.D.
K	23.43	237.4	180.8	203.4	345.1	0.248
Mg	52.89	91.70	109.1	115.1	105.0	41.20
Mn	0.341	0.357	2.58	0.43	0.34	N.D.
Na	61.48	60	62.2	57.94	90	91.62
S	8.74	70.69	9.884	9.18	14.5	0.781
Si	0.675	0.931	1.762	0.713	0.759	N.D.
Sr	183.3	281.2	361.4	353.4	339.7	1.01
Ti	0.079	0.067	0.045	0.067	0.074	0.008
V	0.206	0.267	0.441	0.512	0.457	0.006
W	1.926	3.419	9.343	N.D.	N.D.	3.28
Zn	0.062	0.049	0.054	0.003	0.030	59.02
Zr	0.066	0.073	0.033	0.008	N.D.	N.D.

N.D.: not detected.

SF: pristine lake water from Sterling Forest.

with $1 \times$ PBS to remove any floating cells released during the scratching process. $1 \times$ DMEM was added back into the plates to nourish the cells and photographs were taken at 6 h and 36 h after the scratch using a Leica SP5 microscope (Leica Microsystems, Buffalo Grove, IL). The exact same field of view was captured at each time point with location guidance from the culture plate grid.

Mouse studies

All mouse experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and all efforts were made to minimize suffering. Animals were maintained under protocols approved by the New York University School of Medicine Animal Care

and Use Committee (IACUC). Female athymic nude mice (Nu/J) aged 6–8 weeks were injected subcutaneously with 1×10^7 various transformed BEAS-2B cells and control cells in the left and right flanks to generate solid human tumor xenografts. All mice were housed in specific pathogen free (SPF) environments and observed for tumor growth twice a week. Euthanasia was performed through carbon dioxide overexposure per the American Veterinary Medical Association (AVMA) guidelines.

RNA isolation and RNA-sequencing (RNA-Seq)

Total RNA was isolated from control or treated cells in biological replicates (duplicates for control and triplicates for well water treated samples) and polyA⁺ RNA was purified using poly-T oligo-attached

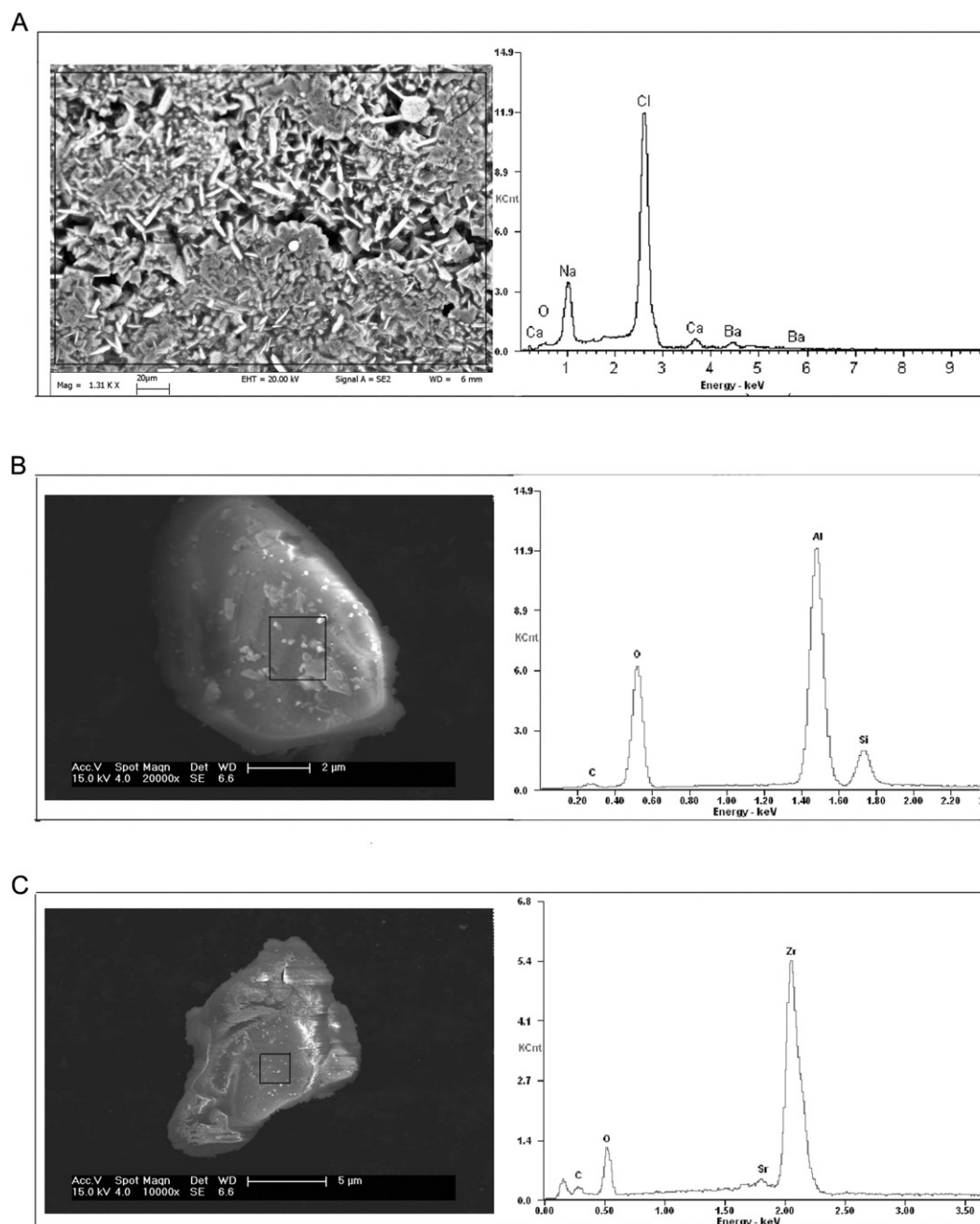


Fig. 1. SEM/EDX analysis of Marcellus flow back water. Representative SEM images reveal the morphology of crystals and particles in flow back samples. The rectangles indicate the regions that were analyzed by the EDX for the composition of the particulates. Samples were prepared at high (A) and low densities (B, C). At low density conditions, individual particles can be isolated and EDX can be performed on each particle.

magnetic beads in accordance to Illumina's protocol for sequencing of mRNA (RNA-Seq). Following purification, the mRNA was fragmented into small pieces using divalent cations under elevated temperature. Then the cleaved RNA fragments were copied into first strand cDNA using reverse transcription and random primers, and followed by second strand cDNA synthesis using DNA Polymerase I and RNaseH. These cDNA fragments were then end repaired, and a single 'A' base was added to allow blunt-end ligation of adapters. These products were then purified and enriched with PCR to create the final cDNA library suitable for high throughput DNA sequencing on the Illumina Cluster Station and Genome Analyzer.

For RNA sequencing, each mRNA sample was uploaded onto one lane of flow cell and sequenced in 36-nucleotide single-end run by Illumina Genome Analyzer II (GAII). For each sample, about 20 million raw reads were generated. Over 90% raw reads were able to be mapped to human genome (GRCh37.71/hg19) by using Bowtie aligner (0.12.9) with v2 and m1 parameters. Mapped reads were subsequently subjected to PCR duplicates removal and reads assignment back to gene model was done by feature counts package. To obtain significant differential expressed genes, the surface water and agar clone experiments were treated as single factor multiple group design while well water experiment was treated as two factor (time and dose) multiple level design. The TMM normalization method in edgeR package (3.4.2) was applied for data normalization cross sample groups before applying general linear model for statistical estimate and analysis for either single factor or two factor designed experiments. FDR adjustment was used for multiple hypothesis test to obtain adjusted p value. A proper FDR cutoff was set to select significant differential expressed genes for subsequent clustering or functional analysis. The results were then validated using quantitative real-time PCR.

Results

High barium and strontium levels in flow back water samples

Elemental analysis from ICP-MS indicated high amounts of strontium (Sr, ranged from 1339 mg/L to 3728 mg/L), and barium (Ba, ranged from 3237 mg/L to 4989 mg/L) beside high sodium (Na, ranged from

28,600 mg/L to 46,100 mg/L) and calcium (Ca, ranged from 6010 mg/L to 15,500 mg/L) levels in these samples (Table S1), which are consistent with the results from the measurements conducted when the water samples were originally collected (Pritz, 2010). Only small amounts of hexacosane and octacosane were detected from three of the five samples (Table S1). To remove any large organics or other confounding biotic before treating the cells, the water samples were filtered through 0.22 μ m polyethersulfone membrane filters and the filtrate was again measured via ICP-MS. As shown in Table 1, Sr and Ba levels remained highly elevated in the filtrates, although substantially reduced from that observed prior to filtration; a phenomenon that was also observed with the other constituents of the samples as well. To characterize the morphological features as well as size parameters of the particles in these samples, SEM/EDX was used. Fig. 1 shows the representative SEM morphology of the particles in flow back water from well 1 (Fig. 1A shows whole water with high density sample preparation, Fig. 1B and C show filtered water with low density preparation). Amorphous silicon aluminum oxide (SiAlOx) nano/micro-particles ranging from 70–285 nm and amorphous zirconium oxide (ZrOx) particles ranging from 40–140 nm, were detected in filtered flow back samples using SEM/EDX analysis, indicating small particle aggregates pass through the 0.22 μ m pore of the filters. The EDX spectrum on individual particles in Fig. 1B and C confirmed the SiAlOx and ZrOx chemistry of the particles but without stoichiometric balancing, the exact oxidation state is not known. Radiation emission detected was not higher than normal background levels in our samples when measured by beta detectors or gamma scintillation counters (data not shown).

Flow back water samples transformed BEAS-2B cells in vitro

BEAS-2B cells were employed as a non-organ-specific assay system to test the transformation activity of the flow back samples. Due to the comparability of components of five flow back samples (shown in Tables S1 and 1), filtered samples from well 3 (representative flow back water after multiple use) and well 1 (representative flow back water after single use) were chosen to treat BEAS-2B cells.

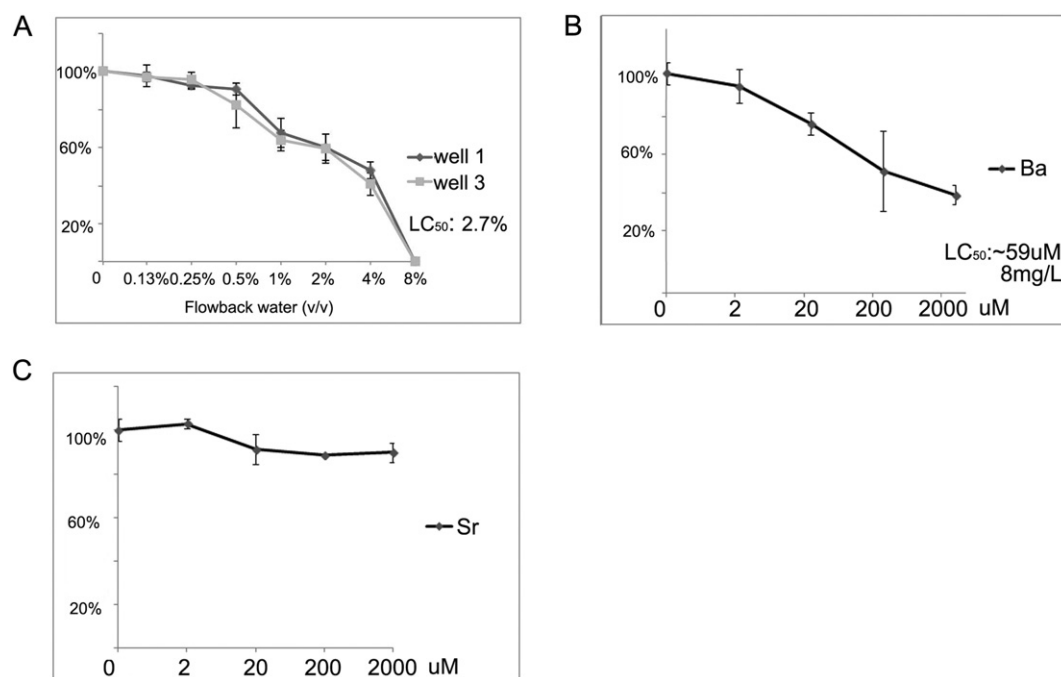


Fig. 2. Cytotoxicity of Marcellus flow back, soluble barium and strontium. The colony formation curve was generated by culturing replated BEAS-2B cells for 16 days after exposed to various concentrations of flow back (A), soluble barium (B) and strontium (C) for 3 weeks (B) and then the number of surviving colonies was determined.

Table 2

ICP-MS analysis of BEAS-2B cells from long-term treatment by Marcellus Shale flow back at 0.5% (v/v).

Concentration ($\mu\text{g}/\text{million cells}$)	Ctrl	Well 1	Well 3	SF
Ba	0.0017	0.1418	0.1378	N.D.
Sr	0.003	0.3941	0.4724	0.044

N.D.: not detected.

SF: cells exposed to filtered pristine lake water from Sterling Forest.

Up to 8% (v/v) dosages were used to assay cytotoxicity. The colony formation assay was carried out to determine an appropriate dose of flow back water samples for treatment of the BEAS-2B cells. This assay was performed with three biological replicates, and the total number of colonies in each replicate fell within two standard deviations of each other. The dose response curve (Fig. 2A) demonstrated that cell survival decreased in a dose-dependent manner. The LC_{50} following treatment for 7 days was calculated to be $\sim 2.7\%$ (v/v). Based on these

data, dosages of 0.13%, 0.25%, 0.5%, 1%, 2%, and 4% were subsequently selected to determine cytotoxicity from long-term water treatment. After 10 days, the BEAS-2B cells treated with 4% flow back failed to survive, while cells treated with the lower doses all survived and were subsequently exposed to flow back water for total of 6 weeks. Soluble barium chloride (Ba II) and strontium chloride (Sr II) were used to determine individual cytotoxicity of each metal. The LC_{50} of Ba (II) following treatment for 7 days was calculated to be $\sim 8 \text{ mg/L}$ (Fig. 2B), this concentration is parallel to the concentrations of Ba when the two studied flow back samples were diluted to 2.7% (12.21 mg/L for well 1 and 16.11 mg/L for well 3), indicating Ba plays a major role in cytotoxicity of Marcellus Shale flow back water. Sr (II) showed no significant cytotoxicity even at the 2 mM concentration in the culture medium (Fig. 2C), which indicated that the cytotoxicity of Ba was not driven by an elevation in osmolality (2 mM SrCl_2 generates same amount of osmolality as 2 mM BaCl_2).

A subset of treated BEAS-2B cells (at 5 week) was collected and the uptake of metals by the cells was then examined by ICP-MS, the metals that increased significantly in flow back water treated cells are shown in

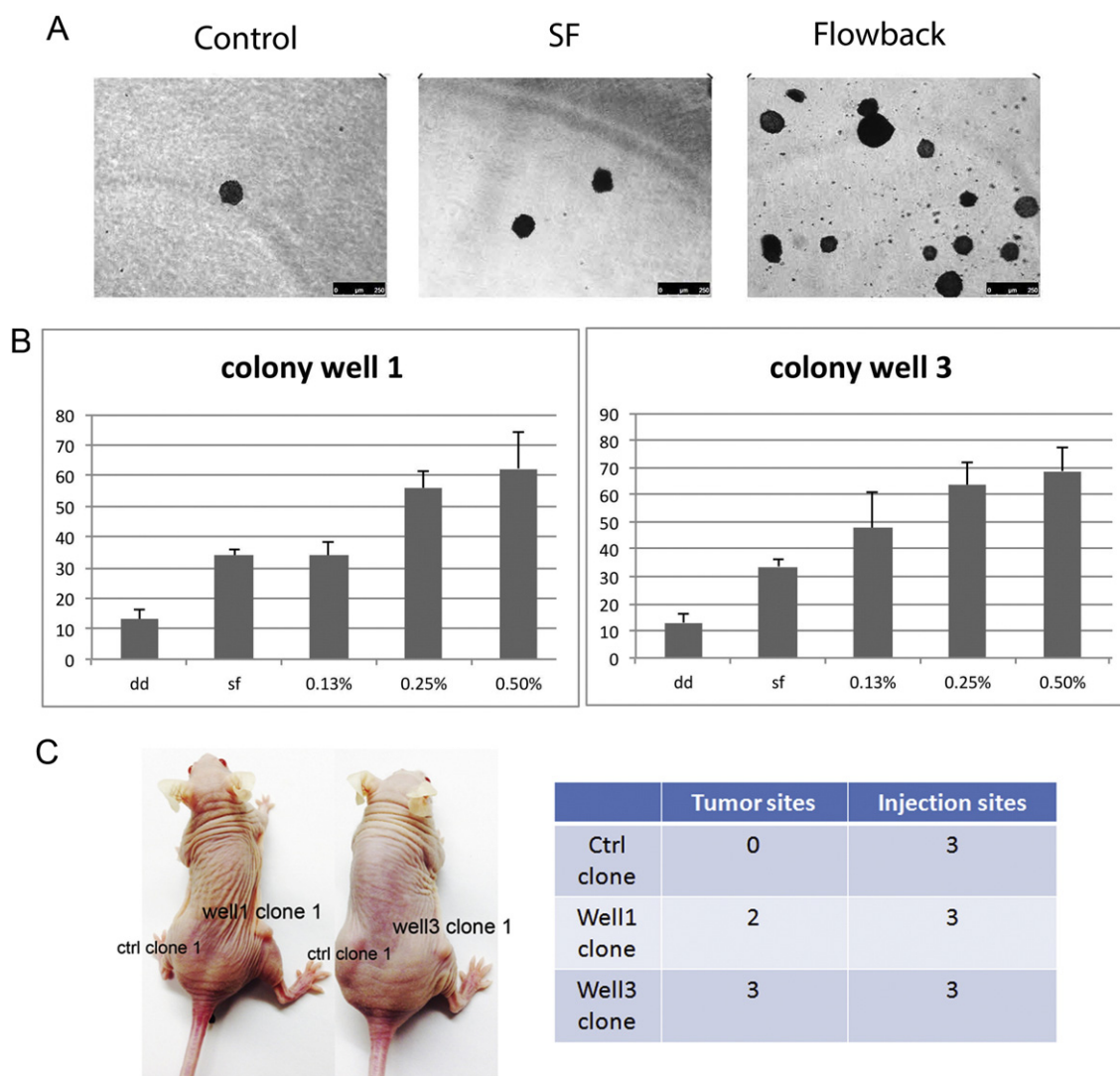


Fig. 3. Anchorage free growth of BEAS-2B cells exposed to Marcellus flow back. BEAS-2B cells were exposed to various concentrations of flow back for 6 weeks, and assessed for anchorage free growth using a soft agar assay. 3 weeks later, cell colonies were stained with INT/BCIP and photographed. Panel A shows representative plates in soft agar assay, control: distilled water, SF: filtered pristine lake water from Sterling Forest. Numbers of colonies formed by BEAS-2B cells exposed to flow back well 1 and flow back well 3 (B) were counted and presented as the mean $\pm$ SD ($n = 3$), dd: distilled water, SF: filtered pristine lake water from Sterling Forest. Panel C shows representative image and the actual number of tumor formation in nude mice after subcutaneous injection of control clones (ctrl clone 1) and flow back water well 1-transformed cells (well 1 clone 1) and flow back water well 3-transformed cells (well 3 clone 1).

Table 2. As expected, Ba and Sr levels increased significantly in cells after long-term treatments when compared to levels in control treated cells (Table 2).

BEAS-2B cells treated with flow back water for 6 weeks were tested for anchorage independent growth using the soft agar colony-forming assay (Fig. 3A and B). The soft agar assay was performed using three biological replicates, and the total number of colonies in each replicate fell within two standard deviations of each other. A dose-dependent increase in colony number in soft agar was observed, with the greatest number of colonies arising from cells treated with 0.5% flow back water recovered from wells 1 and 3 (Fig. 3B). A small number of control cells spontaneously formed colony like clusters in agar; however the numbers were significantly fewer and sizes were considerably smaller (Fig. 3B).

For further transformation studies, the 0.5% (v/v) dose was selected as this dose generated the greatest number of flow back water transformed clones without being cytotoxic. Colonies were then isolated from soft agar, trypsinized and expanded in monolayer culture. Five out of six mice injected with cells transformed from well water treatments developed tumors, while the mice injected with control clones did not form tumors after 6 months (Fig. 3C), indicating the produced flow back water is capable of neoplastic transformation *in vitro*.

After expansion in monolayer culture, clones generated from flow back water treatments exhibited a distinct cell morphology compared to those derived from control clones. As shown in Fig. 4A, control clone cells were flat, diamond-shaped, and similar to their parental BEAS-2B cells, while clones generated from cells treated with flow back water from wells 1 or 3 were rounder, forming a more compact cobblestone-like monolayer. In addition, these clones exhibited a slightly faster growth rate as compared to control cells after 72 h cell culture (Fig. 4B).

Five out of six mice injected with cells transformed from well water treatments developed tumors as early as 3 months after the injection. One mouse carrying xenograft tumor developed from well 1-flow-back-water-transformed-BEAS-2B was sacrificed 5 months after the

injection when the tumor diameter reached 1 cm, others were kept in SPF facility until 6 months after the injection; the tumor diameters ranged from 0.2 cm to 0.6 cm. The mice injected with control clones did not form tumors after 6 months (Fig. 3C); indicating the produced flow back water is capable of neoplastic transformation *in vitro*.

Enhanced migration of flow back water transformed BEAS-2B

After expansion in monolayer culture, clones generated from flow back water treatments exhibited a distinct cell morphology compared to those derived from control clones. As shown in Fig. 4A, control clone cells were flat, diamond-shaped, and similar to their parental BEAS-2B cells, while clones generated from cells treated with flow back water from wells 1 or 3 were rounder, forming a more compact cobblestone-like monolayer. In addition, these clones exhibited a slightly faster growth rate as compared to control cells after 72 h cell culture (Fig. 4B).

Surviving clones from both treatment groups were then assayed for cell migration. Matrigel assay showed that BEAS-2B cells transformed by flow back well water samples presented an enhanced migration capacity than control cells (Fig. 5A). In line with the results from Matrigel assay, all of the clones generated from flow back water treatments were able to heal the wound by 36 h post-scratch while control clones failed to heal the wound in the same amount of time (Fig. 5B).

Altered transcription profile in flow back water transformed BEAS-2B

We analyzed transcription profiles of transformed cells by sequencing the RNA libraries prepared from clones generated from flow back water treatments and control clones (Fig. 6). Multidimensional scaling and hierarchical clustering dendrogram revealed that transformed clones cluster according to their treatment group. The transcription profiles were then analyzed by DAVID Bioinformatics Resources 6.7 (National Institute of Allergy and Infectious Diseases, NIH). The differentially expressed genes have been listed in Table S3 (well 1 clones Vs ctrl

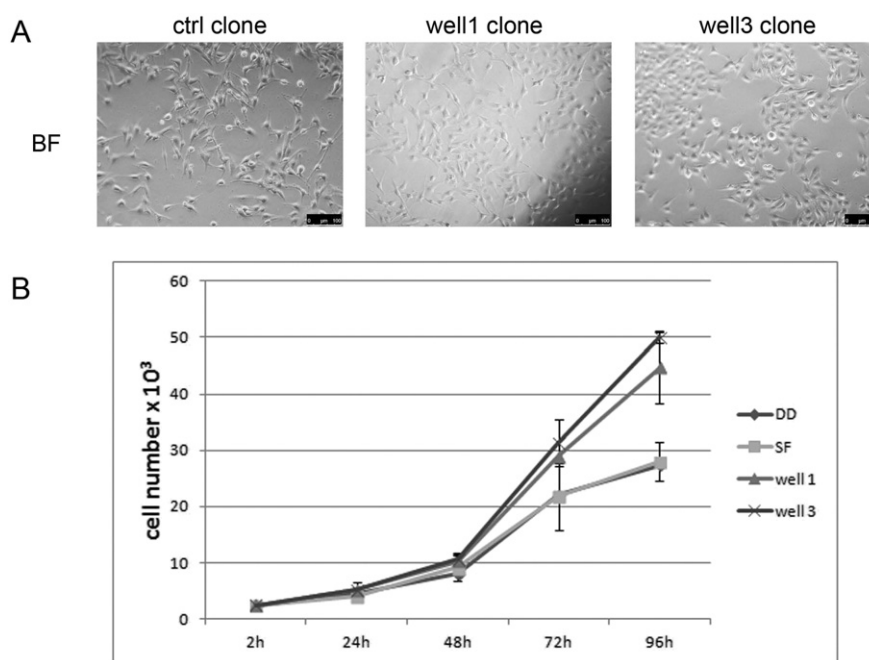


Fig. 4. Analysis of transformed cells derived from soft agar. (A) Representative image of normal BEAS-2B cells derived from spontaneously derived colonies of untreated cells (ctrl clone), flow back well 1 transformed cells (well 1 clone) or flow back well 3 transformed cells (well 3 clone) grown in low density. (B) Control and flow back transformed cells were seeded at 2500 cells/well in 24-well plates. Cells were trypsinized and counted at the indicated time point. Results were represented as mean $\pm$ SD (n = 3), DD: distilled water, SF: filtered pristine lake water from Sterling Forest.

clones) and Table S4 (well 3 clones Vs ctrl clones). For DAVID analysis, differentially expressed genes (Table S5) that are common in both well 1 and well 3 clones were used. The genes with up-regulated expression in transformed clones were over-presented by inflammation, cell migration, cell proliferation and *wnt* signaling pathway (Fig. 6C), while down-regulated genes were over-presented by adherens junction, apoptosis, and endocytosis (Fig. 6D), which are consistent with the phenotypes we observed in transformed clones (Figs. 4 and 5).

The transcription factor binding motifs overrepresented at the differentially expressed genes' promoter sequences (−250 and +50 base pairs around the transcription start site) were identified using Opossum 3.0 with JASPAR CORE transcription factor binding profiles. The conservation cutoff was set at 0.6 and only those transcription binding sites with Z-score ≥ 10 and Fisher score ≥ 7 were shown in Fig. 6E (transcription binding sites associated with up-regulated genes) and Table S6 (transcription binding sites associated with down-regulated genes). Interestingly, the over-represented conserved transcription binding sites in up-regulated genes (Fig. 6E) are of transcription factors that are known to associate with cancer (SP1, HIF-1 α , MZF1) and stem cell self-renewal (KLF4, NFY α).

Discussion

The objective of this study was to investigate the potential cytotoxicity and transforming activity of Marcellus Shale well flow back water to mammalian cells. To the best of our knowledge, this is a first report of this nature. Human and animal exposure to flow back water occurred through leakage or improper fencing of impoundments, and/or via alleged compromise of a liner in an impoundment to drain fluid, direct discharging of the flow back water to the creeks and nearby land (Bamberger and Oswald, 2012).

Ba and Sr appeared to be metals with high concentrations in these aged flow back samples that were found elevated in cells after long-term treatment. There are reports showing arsenic and selenium are constituents of gas containing rock bed (Haluszczak et al., 2013; Jackson, 2013), however, these elements were below the detection limit of ICP-MS in these specific five flow back water samples. Based on this study and previous report (Jackson, 2013), the high Ba and Sr levels are not likely due to fracking procedure but rather the nature of Marcellus Shale. Sr and Ba are two alkaline elements that mimic calcium in the body of living organisms, therefore high concentration of Ca will reduce the absorption of these two metals (Comar et al., 1957). In these

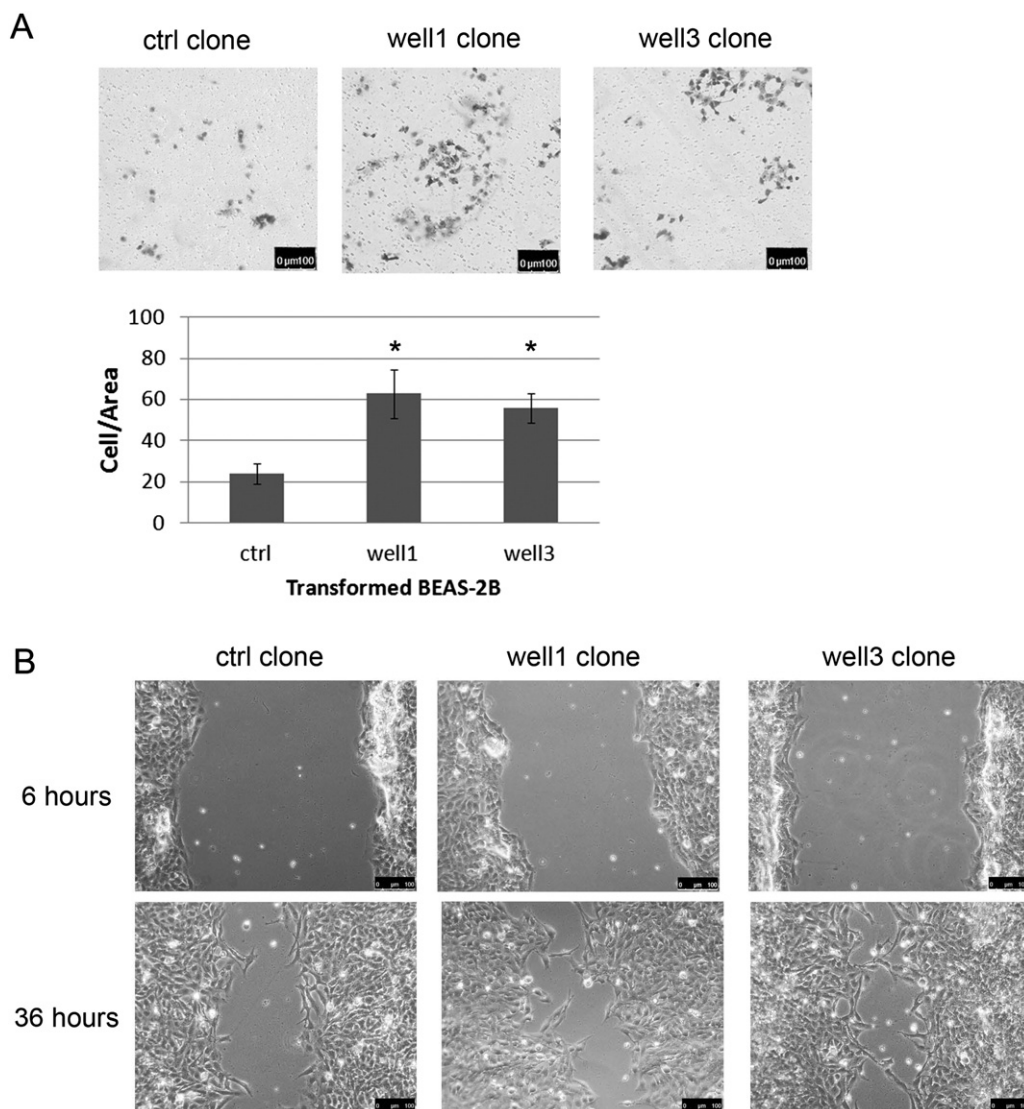


Fig. 5. Enhanced cell migration of transformed BEAS-2B. (A) Representation (top) and quantification (bottom) of Matrigel invasion assay showing the in vitro migration of transformed BEAS-2B cells. * $p < 0.05$ (B) flow back transformed clones healed the wound faster than the control clones. All images were captured at 100 $\times$ magnification, and the same field of view was captured at each time point with guidance from the grid on the cell culture plates.

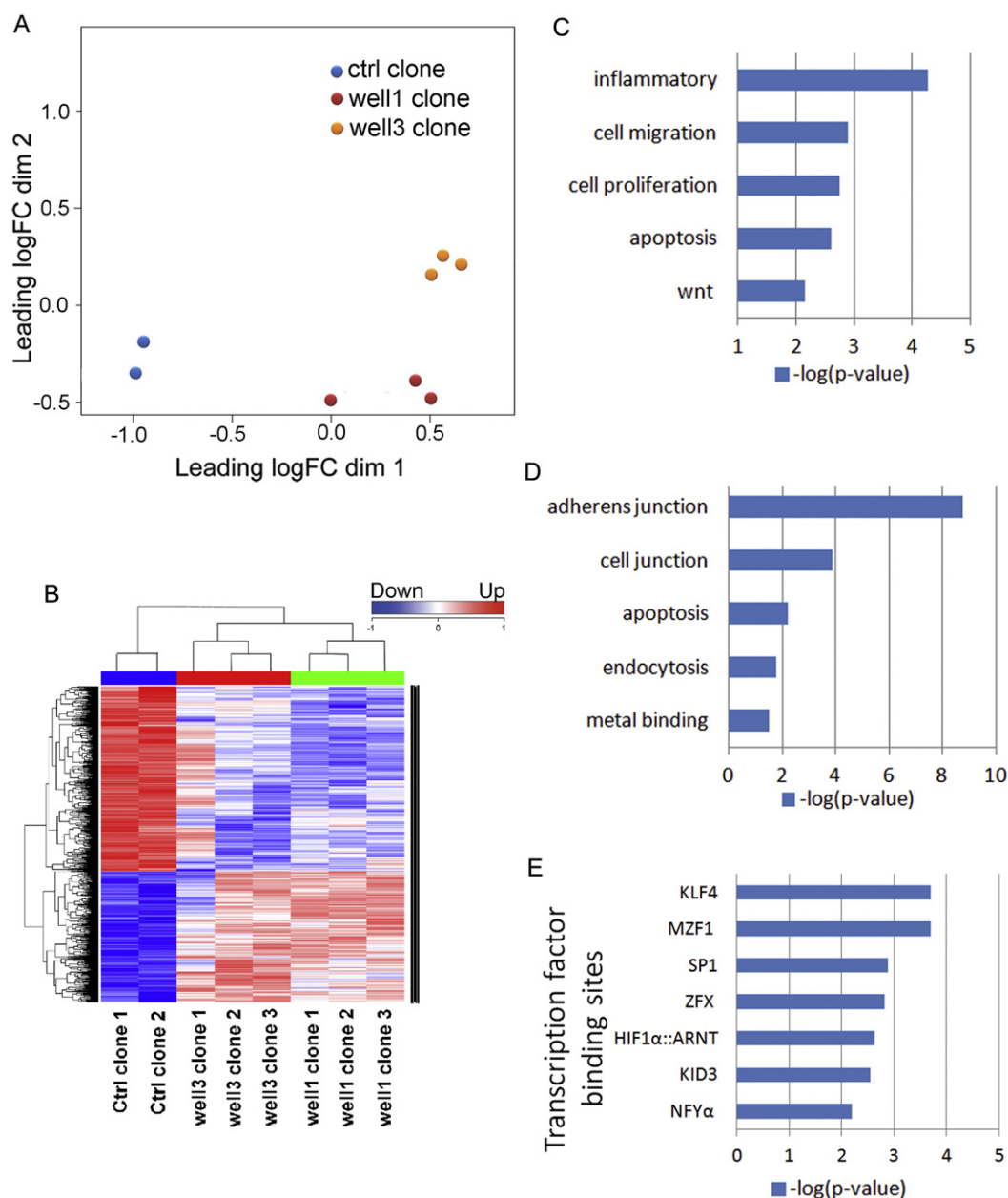


Fig. 6. RNA-Seq analysis of transformed BEAS-2B cells. (A) Hierarchical clustering dendrogram shows 1298 genes resulting from a multiple hypothesis test, FDR < 0.05, for all 8 clones (6 flow back water-transformed clones, 2 control clones). Genes that were increased in expression compared to their control clones are shown in red while under-expressed genes are depicted in blue. (B) A multidimensional scaling plot shows that clones derived from individual flow back water wells treated cells generally cluster together. (C and D) GO analyses showing the major canonical pathways associated with the genes (C) upregulated and (D) downregulated in flow back well water sample transformed BEAS-2B cells. (E) Identification of the overrepresented transcription factor binding motifs at the promoters of genes upregulated in flow back well water sample transformed BEAS-2B cells.

flow back water samples, we found high concentration of Ca, however, after long-term treatment, the absorption of Ba and Sr was still detectable by ICP-MS.

Although the World Health Organization (WHO) 4th edition of health-based guidelines sets 700 µg/L as a guideline for Ba levels in drinking water, a lower level (i.e., 343.3–686.6 µg/L) of Ba alone promotes transforming activity of several cell lines (Thang et al., 2011). It's notable that the concentrations of Ba in the flow back water we used were at 2262 µg/L (well 1) and 2982.5 µg/L (well 3). In Iran, Sr concentrations in drinking water, soil, and grain samples are much higher (3437.3 µg/L) in areas with a high esophageal cancer incidence rate (i.e., Gonbad-Dashlibroon and Marave Tappeh regions) compared to those in areas where the esophageal cancer incidence is lower (Keshavarzi et al., 2012a; Keshavarzi et al., 2012b), suggesting adverse

impacts of Sr to human health. Notably, the concentrations of Ba and Sr in our whole flow back water samples are at least a thousand-fold higher than concentrations in the drinking water from these areas, making direct discharge of flow back to surface water body a pressing water and soil pollution problem, which in turn could affect the health of animals and humans in close proximity. Furthermore, we believe the major components in the aged water samples that we tested in this study are also present in conventional gas drilling flow back waters. Our work suggested the importance of proper wastewater regulation and treatment before its discharge to surface water, and it is applicable to both conventional and unconventional gas drillings.

In response to concern over flow back and produced water discharges, the Pennsylvania Department of Environmental Protection (PADEP, Pennsylvania is the state where the wastewater in this study

was originated from) proposed a new strategy that would add effluent standards for oil and gas wastewaters of 500 mg/L for total dissolved solids, 250 mg/L for sulfates, 250 mg/L for chlorides, and 10 mg/L for total Ba and total Sr (Veil, 2010). Moreover, the Pennsylvania Environmental Quality Board approved the new discharge requirements as revisions to the Pennsylvania regulations (Veil, 2010). As the Marcellus Shale development grows in popularity, operators seek to bring more truckloads of salty flow back and produced water to the treatment plants. In this case, it is most likely that the increased input of total dissolved solids will result in increased levels of total dissolved solids in the discharge. Close monitoring and restrictions on the discharge should be conducted to limit the impact on animal and human health. National pollutant discharge elimination system that requires that the volume of wastewater from oil and gas sources may not exceed 1% of the average daily flow has been adopted by many of the water treatment works (Veil, 2010); whether this regulation is sufficient to prevent the environmental impact on the ecosystem warrants further investigation.

The flow back water used in this study while transformative may not be truly representative as it was aged prior to the physical–chemical characterization necessary in this set of experiments; and thus neither significant amount of radioactivity or organic compounds was present. While the time of laboratory storage accounted for changes in the chemistry of the samples, it could be noted that some wastewater generated from hydraulic fracturing has been stored in disposal pits for years and may therefore be more representative of the long term hazards posed by improper disposal or containment. Results from this study suggest that even aged flow back water could pose substantial health threats to exposed humans. The absence of volatile organic compounds aided in the identification of toxicity due mostly from Ba, Sr, or other metals in the flow back waters. Due to limited resource, we were not able to measure Radium (Ra226 and/or Ra228 which have been reported in some of the other Marcellus Shale flow back water samples (Pritz, 2010). Ra226, an alpha emitter, has a half-life of about 1600 years with accompanying gamma radiation; and Ra228, a beta emitter, has a half-life of 5.76 years). We attempted to determine whether there was any gamma radiation emitted from the water using a gamma counter but we did not detect any radiation. The measurements of metals in the filtrates and cells exposed to the filtrates helped us to assess the presence of toxic metals and their relative concentrations.

The cells transformed by flow back water formed tumors in athymic nude mice and exhibit higher migration ability in transwell assay and wound healing assay. These phenomena have been further supported by gene expression analysis. Cell migration pathways are up-regulated and adherent junction pathways are down-regulated in flow back water transformed cells. This is a common phenomenon for malignant cell transformation which we have reported earlier nickel, arsenic or vanadium transformed BEAS-2B cells (Clancy et al., 2012). However, the transcription profile alteration induced by flow back water which contains various kinds of elements still has its distinguish signature. The genes with increased expression level in flow back water transformed cells are over-represented by inflammation, while the down-regulated genes are over-represented by endocytosis; indicating the cells went through an inflammation reaction and a reduction of endocytosis to cope with elevated metal levels in their micro-environment upon their encounter with metal enriched flow back water. However, based on the data we gathered, we are not able to tell whether the malignant transformation activity was solely from Ba, Sr or other metals that have been detected in the flow back waters at lower levels.

It is worth noting that binding sites of a few transcription factor that are associated with stem cell self-renewal (KLF4, NFYα) and cancer (SP1, HIF-1α, MZF1) are found to be over-represented in genes with up-regulated expression in flow back water transformed cells. MZF1 (myeloid zinc finger 1) is also up-regulated at its gene expression level. The overexpression of MZF1 was reported to inhibit apoptosis

and induce migration, invasion, tumor formation, and metastasis in vivo in cultured colorectal and cervical cancer cells (Mudduluru et al., 2010). Our expression analysis against transformed cells provided a potential molecular mechanism of how flow back water transforms human cells at gene expression level. In addition, it has been previously shown that there is a fingerprint of gene expression in transformed clones that is characteristic for each metal that was used to induce the anchorage independent growth (Clancy et al., 2012). This seems to be also true for the flow back water induced transformation and this finding argues that Ba and perhaps Sr were the major carcinogenic species in the flow back water.

Conclusions

Our work has provided the first line of evidence that Marcellus Shale flow back water induces malignant cell transformation in vitro. The BEAS-2B cells exposed to flow back water up to six weeks appeared to be transformed and exhibiting altered morphology as compared to parental cells. The present work also provided Ba and Sr as hydraulic fracturing-related target pollutants in addition to the more classically-studied fracking contaminants (i.e., radioisotopes and methane) for further investigation. Research to determine whether fracking-associated pollutants can migrate to private or public drinking wells, to identify early warning indicators of exposure and effect, and to identify suitable remediation approaches are urgently needed. Descriptive and analytical epidemiological studies along with animal model studies will help to better understand the health impact associated with unconventional shale gas production.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.taap.2015.07.011>.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Estimating the Potential Toxicity of Chemicals Associated with Hydraulic Fracturing Operations Using Quantitative Structure–Activity Relationship Modeling

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S Supporting Information

ABSTRACT: The United States Environmental Protection Agency (EPA) identified 1173 chemicals associated with hydraulic fracturing fluids, flowback, or produced water, of which 1026 (87%) lack chronic oral toxicity values for human health assessments. To facilitate the ranking and prioritization of chemicals that lack toxicity values, it may be useful to employ toxicity estimates from quantitative structure–activity relationship (QSAR) models. Here we describe an approach for applying the results of a QSAR model from the TOPKAT program suite, which provides estimates of the rat chronic oral lowest-observed-adverse-effect level (LOAEL). Of the 1173 chemicals, TOPKAT was able to generate LOAEL estimates for 515 (44%). To address the uncertainty associated with these estimates, we assigned qualitative confidence scores (high, medium, or low) to each TOPKAT LOAEL estimate, and found 481 to be high-confidence. For 48 chemicals that had both a high-confidence TOPKAT LOAEL estimate and a chronic oral reference dose from EPA's Integrated Risk Information System (IRIS) database, Spearman rank correlation identified 68% agreement between the two values (permutation p-value = 1×10^{-11}). These results provide support for the use of TOPKAT LOAEL estimates in identifying and prioritizing potentially hazardous chemicals. High-confidence TOPKAT LOAEL estimates were available for 389 of 1026 hydraulic fracturing-related chemicals that lack chronic oral RfVs and OSFs from EPA-identified sources, including a subset of chemicals that are frequently used in hydraulic fracturing fluids.



■ INTRODUCTION

Hydraulic fracturing is a stimulation technique used to increase production of oil and gas from geological formations, especially from unconventional reservoirs such as shale, tight sands, and coalbeds. Large volumes of pressurized fluids are injected into subsurface wells to fracture the hydrocarbon formation, resulting in the release of oil or gas.^{1,2} While this practice has enabled an increase in oil and gas production in the United States, there are concerns about potential public health implications, including the potential for hydraulic fracturing-related chemicals to impact drinking water resources.^{1–6}

Chemicals associated with hydraulic fracturing activity include gelling agents, breakers, surfactants, corrosion inhibitors, and others, which are used as additives in hydraulic fracturing fluids.^{7–9} Other chemicals, such as naturally occurring organic and inorganic compounds, may be mobilized from the formation during drilling and hydraulic fracturing activity. This mixture of chemical additives and chemicals from the formation may return to the surface in flowback and produced water from the well.^{10,11} “Produced water” is a general term used to refer to water that flows from oil and gas wells, which may include hydraulic fracturing fluids as well as

natural waters from the formation. “Flowback” is a type of produced water, and refers to fluids containing predominantly hydraulic fracturing fluids that return to the surface after the pressure on a well is initially released. Flowback and produced water are generally stored in open air impoundments or storage containers at the well site, and may be recycled, treated for release into waterways, or disposed of in underground injection wells.^{12–14} To date, a lack of exposure assessment data and systematic studies makes it difficult to assess the extent to which people may be exposed to these chemicals in hydraulic fracturing fluids, flowback, and produced water via contamination of drinking water resources.^{6,15} However, there are specific instances in which hydraulic fracturing activities have been implicated in the contamination of groundwater and surface water. Events that may lead to contamination include surface spills, leaks from flowback/produced water storage pits, and well blowouts.^{16–20}

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There have been efforts to compile data on the toxicity of chemicals in hydraulic fracturing fluids, flowback, and produced water. The U.S. Environmental Protection Agency (EPA) recently released the external review draft of the *Assessment of the Potential Impacts of Hydraulic Fracturing for Oil and Gas on Drinking Water Resources*,²¹ which identified a list of 1173 chemicals that have been associated with hydraulic fracturing operations nationwide. This list includes 1076 chemicals used as additives in hydraulic fracturing fluids, and 134 chemicals reported in flowback or produced water. Using selected high-quality data sources, EPA compiled toxicity values for these chemicals when available, including chronic oral reference values (RfVs) for noncancer toxicity and oral slope factors (OSFs) for cancer. These values are commonly used by risk assessors to evaluate hazards of long-term chemical exposure via drinking water. Chronic oral RfVs estimate the amount of chemical that can be ingested daily by the human population (including sensitive subgroups) that is likely to be without appreciable risk of health effects over a lifetime.²² OSFs are the upper bound on increased cancer risk from lifetime oral exposure to a chemical.²² We recently performed an analysis of EPA's data compilation,²³ and reported that the selected chronic oral RfVs or OSFs are available for 90 (8%) of the chemicals used in hydraulic fracturing fluids, and 83 (62%) of chemicals reported in flowback/produced water. Of the 36 chemicals used in hydraulic fracturing fluids in at least 10% of wells nationwide, eight chemicals (22%) have a chronic oral RfV. Overall, chronic oral RfVs or OSFs were lacking for 1026 (87%) chemicals on EPA's total list, suggesting a significant data gap for risk assessment.²³ In a similar vein, other studies in the peer-reviewed literature have found that relevant toxicity data is often lacking for chemicals used in hydraulic fracturing fluids.^{5,9,24,25}

To facilitate the ranking and prioritization of potentially hazardous chemicals at hydraulic fracturing sites, alternative approaches may be used when toxicity values like RfVs or OSFs are not available. Quantitative structure–activity relationship (QSAR) models are one such approach that may provide useful information. QSAR toxicity models operate on the assumption that molecular structure or other chemical properties can be adequately correlated to biological activity. The models are developed using a “training set” of selected chemicals that have experimentally derived toxicity data, and employ statistical relationships (regression or classification models) to estimate toxicity for new chemicals of interest based on similarities in their structure and properties to the training set.²⁶ A key advantage of QSAR models is that they require only a chemical structure, and can allow for the estimation of toxicity on thousands of chemicals at once, making these models amenable to situations where empirical data are not available.^{26–29} A disadvantage is that QSAR models are generally considered to have more uncertainty than animal models.^{27,28}

To our knowledge, TOPKAT (“Toxicity Prediction by Komputer Assisted Technology”) is the only commercially available QSAR software capable of estimating a quantitative value related to the point of departure (POD) for toxicity—in this case, the rat chronic oral lowest-observed-adverse-effect level (LOAEL). The LOAEL is defined as the lowest exposure level at which there are biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control group.²² Unlike RfVs, LOAELs are not health-protective values, because they describe a dose at which effects are known to occur. However, chronic

oral LOAELs are useful for ranking chemicals based on potential toxicity, and are relevant for evaluating hazards due to long-term chemical exposure via drinking water. We are interested in evaluating the feasibility of using TOPKAT LOAEL estimates to evaluate the potential toxicity of chemicals associated with hydraulic fracturing activity, as risk assessors or researchers may choose to employ these estimates if they are faced with numerous data-poor chemicals at a field site.

Here, we use TOPKAT to generate LOAEL estimates for EPA's list of chemicals in hydraulic fracturing fluids, flowback, and produced water, and describe an approach for using these estimates to rank and prioritize these chemicals based on potential toxicity. To address some of the limitations and uncertainties in using QSAR models, we develop and apply a framework for assigning qualitative confidence levels (high, medium, low) to the TOPKAT LOAEL estimates. We then verify the ability of high-confidence TOPKAT LOAEL estimates to rank these chemicals based on relative hazard, and examine how many high-confidence TOPKAT LOAEL estimates are available for chemicals on EPA's list that lack chronic oral RfVs and OSFs. In keeping with the scope of EPA's hydraulic fracturing study, our analysis is focused on toxicity values for oral exposure via drinking water; toxicity values for other potential routes of exposure to these chemicals (e.g., inhalation, dermal) are not evaluated here. Risk assessors and researchers may use this approach in combination with chemical exposure data from specific field sites, in order to create an overall risk ranking and assist in public health decision making.

MATERIALS AND METHODS

EPA's List of Hydraulic Fracturing-Related Chemicals and Associated Toxicity Values. EPA's identification of hydraulic fracturing-related chemicals and their associated toxicity values was described in the external review draft of the *Assessment of the Potential Impacts of Hydraulic Fracturing for Oil and Gas on Drinking Water Resources*,²¹ and was reviewed in the concurrent study by our group.²³ Briefly, EPA used ten sources of information to compile a list of 1173 chemicals associated with hydraulic fracturing fluids, flowback, or produced water. This list represents a nationwide assessment; individual sites will likely have only a small fraction of the chemicals on this list, and may have additional chemicals that were not identified on this list.

EPA identified toxicity values when available for these 1173 chemicals, using six selected data sources: EPA's Integrated Risk Information System (IRIS) database, EPA's Provisional Peer-reviewed Toxicity Value (PPRTV) database, EPA's Human Health Benchmarks for Pesticides (HHBP) database, the Agency for Toxic Substances and Disease Registry (ATSDR), the World Health Organization Concise International Chemical Assessment Documents (CICAD), and the California Environmental Protection Agency (CalEPA) Toxicity Criteria Database. These data sources were selected because they met criteria set forth by the EPA for the purpose of this study, as described in Appendix G in the draft *Assessment of the Potential Impacts of Hydraulic Fracturing for Oil and Gas on Drinking Water Resources*.²¹ From these six sources, chronic oral RfVs and OSFs were cumulatively available for 147 (13%) of the total 1,173 chemicals. The complete list of chemicals and toxicity values and data sources was finalized as of June 4, 2015, and is available at EPA's draft database for this study (<http://cfpub.epa.gov/ncea/hfstudy/recordisplay.cfm?deid=308341>).³⁰

QSAR Toxicity Estimation using TOPKAT. We used v3.1 of the TOPKAT LOAEL model (implemented via Accelrys Discovery Studio 3.5, 2012) to generate LOAEL estimates for EPA's list of hydraulic fracturing-related chemicals. This version of the TOPKAT LOAEL model was developed using a training set of 393 chemicals (including acyclics, alicyclics, hetero-aromatics, single benzenes, and multiple benzenes) with experimentally derived rat chronic oral LOAELs from National Toxicology Program technical reports, EPA databases, and the scientific literature. The LOAELs used in the training set may be based on a variety of biological endpoints, including hepatotoxicity, reproductive/developmental toxicity, neurotoxicity, and immunotoxicity. TOPKAT does not disclose which data were used in the training set, nor does it report which biological endpoint is associated with the LOAEL estimate reported by the model. Additional information on the TOPKAT LOAEL model is available from previously published studies.^{27–29}

Due to modeling restrictions, LOAELs can be estimated only for organic chemicals whose structures were desalted. TOPKAT was able to estimate LOAELs for 515 (44%) of the 1173 hydraulic fracturing-related chemicals on EPA's list. The remaining chemicals were not able to be modeled due to the nature of their chemistry (e.g., inorganic salts, metals, metal-containing organics, and others) or the fact that they were mixtures (e.g., petroleum distillates, guar gum) or polymers.

For these 515 chemicals, we additionally used the TOPKAT LD₅₀ model to estimate rat oral LD₅₀s. The LD₅₀ is the dose that results in death for 50% of the tested animals. The TOPKAT LD₅₀ model is based on a training set of 4000 chemicals, and consists of 19 submodels that use molecular structure to perform predictions. The TOPKAT LD₅₀ estimates were used in the qualitative confidence analysis, described in the text below.

The full list of 515 chemicals and their associated TOPKAT LOAEL estimates, LD₅₀ estimates, and qualitative confidence scores can be found in the [Supporting Information Table](#) in the tab "TOPKAT LOAELS".

Qualitative Confidence Analysis. We developed a method to determine the qualitative confidence (high, medium, or low) associated with the TOPKAT LOAEL estimates. These confidence scores are based primarily on the structural similarities between a query chemical and the training set that was used for TOPKAT LOAEL model development. This is an appropriate metric for establishing confidence, as a QSAR model can only be expected to give reliable predictions for chemicals that are similar to those used to develop the model.³¹ The confidence scores also take into account the relationship between the TOPKAT LOAEL estimate and the TOPKAT LD₅₀ estimate, which is another metric that warrants consideration as part of the overall weight of evidence. The following three scores were developed and then summed to calculate a composite score:

Optimum Prediction Space (OPS) Score. OPS is the measure used by TOPKAT to assess the applicability domain of the model, which is the chemical space in which the model is expected to be able to make predictions. The chemical space is defined as the universe of chemical structures that were used to train the model. If the structure of a query chemical is similar to those within the universe, then it is likely within the OPS of the model. We assigned a score of 2 when the chemical was within the OPS, a score of 1 when the chemical was within the OPS with the exception of a marginal value (i.e., is near the edge of

the OPS, as defined by TOPKAT), and a score of 0 when the chemical was likely outside of the OPS.

Unknown Fragments (UFS) Score. This score is based on chemical fragments, which are the chemical substructures used as parameters in the TOPKAT LOAEL model. A chemical fragment may be as simple as a methyl group, or as complex as a hydroxylated ring structure with an R-group or a branched halogenated trans-alkene. Unknown fragments are those not represented within the model's training set, and may alter biological activity in ways that cannot be predicted by the model. It is possible for a chemical to be within the OPS while also having a fragment that is not otherwise known or present within the training set. We assigned a score of 2 when a chemical has 0 unknown fragments, a score of 1 when a chemical has 1 unknown fragment, and a score of 0 when a chemical has 2 or more unknown fragments.

LD₅₀ Score. By definition, the LD₅₀ should be greater than the LOAEL. We assigned a score of 1 if the TOPKAT LD₅₀ estimate was greater than the TOPKAT LOAEL estimate. Otherwise, we assigned a score of 0.

The composite score was calculated as the sum of the OPS Score, UFS Score, and LD₅₀ Score. Thus, chemicals with the highest confidence are within the chemical space of the model's training set (OPS Score), have no unknown fragments (UFS Score), and have a TOPKAT LD₅₀ estimate that is greater than the TOPKAT LOAEL estimate (LD₅₀ Score). Composite scores of 4 or 5 are deemed high-confidence, a score of 3 is deemed medium-confidence, and a score of 0, 1, or 2 is deemed low-confidence.

QSAR LOAEL-Based Rank Verification. To evaluate the suitability of TOPKAT LOAEL estimates for chemical ranking and prioritization, a comparison was made between a ranking based on high-confidence TOPKAT LOAEL estimates and a ranking based on IRIS chronic oral reference doses (RfDs), when these two values were available for those same chemicals. IRIS chronic oral RfDs are one of the RfVs identified by EPA during the compilation of toxicity values for the hydraulic fracturing study.²³ We chose IRIS chronic oral RfDs for this comparison because they enabled us to focus on the largest subset of toxicity values that were derived using a consistent approach; toxicity values from multiple sources would introduce a higher level of uncertainty and variability into our analysis. IRIS chronic oral RfDs are typically derived starting with a POD that was observed in humans or in an animal model—generally, a LOAEL, no-observed-adverse-effect level (NOAEL), no-observed-effect level (NOEL), or benchmark dose lower confidence limit (BMDL)—and applying uncertainty factors of up to several orders of magnitude to derive a value for the protection of human health.³²

For the comparison of rank orders, we identified all chemicals that had both a high-confidence TOPKAT LOAEL estimate and an IRIS chronic oral RfD, and ranked the chemicals (i.e., ordered from most toxic to least toxic) according to each of these values. IRIS had chronic oral RfDs for 77 of the 1173 chemicals, of which there were high-confidence TOPKAT LOAEL estimates for 51 of these. To prevent a potential overlap between the TOPKAT LOAEL model training set and the PODs used to derive IRIS chronic oral RfDs, we excluded from this analysis all IRIS chronic oral RfDs that were derived from rat LOAELs ($n = 3$). Such an overlap would confound the correlation analysis. The values for the remaining 48 chemicals were used in calculating a Spearman rank correlation (R version 3.2.0) to evaluate the

similarity between the two rank orderings. In order to determine statistical significance, we followed this with a permutation test, in which we performed the Spearman rank correlation 100 000 times with the chemical ranks randomly assigned in the TOPKAT and IRIS groups (R version 3.2.0). By randomly assigning the ranks, we constructed a distribution of the Spearman correlation values that could occur by chance for the chemicals.

As an additional and more conservative measure to ensure against potential confounding, the correlation analysis was then repeated using only those chemicals that were not present in the TOPKAT LOAEL model training set ($n = 27$). The concern here is that there could be statistical correlation between RfDs and the LOAELs used in the training set, even if these values were derived from different species. TOPKAT does not provide a list of chemicals that were used in the training set, but we were able to identify these chemicals based on the “similarity distance” reported in the output of each TOPKAT LOAEL estimate. Similarity distance is an indicator of how similar a chemical structure is to the chemicals that were used in the TOPKAT training set, and a similarity distance score of 0 indicates that a chemical is an exact match for a chemical in the training set.

For the Spearman rank correlations, TOPKAT LOAEL estimates were rounded to three significant figures. Further rounding would have led to numerous ties in the rank ordering, which would change the correlation results. Therefore, in order to reflect the model output and maintain a reasonable estimate of the rank ordering, we did not round the TOPKAT estimates further. Moudgal et al.²⁹ similarly report TOPKAT LOAEL estimates at 3–4 significant figures.

QSAR-Informed Prioritization of Hydraulic Fracturing-Related Chemicals. We assessed how many high-confidence TOPKAT LOAEL estimates were available for the chemicals on EPA’s list that had neither a chronic oral RfV nor OSF available. The full list of chemicals that had TOPKAT LOAEL estimates, but for which a chronic oral RfV or OSF was not available, can be found in the [Supporting Information Table](#) in the tab “TOPKAT ranked chemicals”. Within this tab, chemicals are ordered from highest to lowest in terms of estimated toxicity.

In our concurrent study,²³ we analyzed the project database of EPA’s *Analysis of Hydraulic Fracturing Fluid Data from the FracFocus Chemical Disclosure Registry 1.0*,³³ and identified a list of 36 chemicals that were reported in at least 10% of disclosures on the database. The FracFocus Chemical Disclosure Registry 1.0 (“FracFocus 1.0”) is a national hydraulic fracturing chemical registry developed by the Ground Water Protection Council and the Interstate Oil and Gas Compact Commission, and was one of the ten data sources used to compile the list of chemicals for EPA’s hydraulic fracturing study. “Disclosure” refers to all data submitted for a specific oil or gas well for a specific fracture date, including data on chemical usage. Here, we assessed the availability of TOPKAT LOAEL estimates for this list of frequently used chemicals from FracFocus 1.0.

■ RESULTS AND DISCUSSION

TOPKAT LOAEL Estimation. Overall, TOPKAT was able to generate LOAEL estimates for 515 (44%) of the total 1173 hydraulic fracturing-related chemicals identified by EPA. Of the 1076 chemicals used in hydraulic fracturing fluids, TOPKAT was able to generate estimates for 453 (42%). Of the 134 chemicals reported in flowback/produced water, TOPKAT was

able to generate estimates for 86 (64%). TOPKAT LOAEL estimates for chemicals used in hydraulic fracturing fluids ranged from 1.50 mg/kg [*N*-(3-Chloroallyl)hexaminium chloride] to 4,040 mg/kg [Di(2-ethylhexyl) phthalate]. TOPKAT LOAEL estimates for chemicals in flowback/produced water ranged from 0.442 mg/kg-day (dieldrin) to 4740 mg/kg (dioctyl phthalate). Note that lower LOAEL values indicate greater toxicity.

It is important to keep in mind that EPA’s list represents a nationwide assessment, encompassing the range and variety of chemicals that may be present in hydraulic fracturing fluids, flowback, or produced water.²³ Some chemical additives are known to be used frequently in hydraulic fracturing fluids—for instance, see our analysis of frequently used chemicals, below. For other chemicals, the frequency of use is unclear. Many chemicals have some reported use in hydraulic fracturing fluids, but were not identified in EPA’s analysis of FracFocus 1.0, due in part to differences in reporting requirements between states.⁷ Within FracFocus 1.0, industry operators were not required to disclose chemicals claimed as confidential business information (CBI).⁷ The use of CBI likely limits the completeness of EPA’s chemical list.

Regarding flowback/produced water, some chemicals on EPA’s list are known to be widely detected. Examples include BTEX (benzene, toluene, ethylbenzene, xylenes), naphthalene, and related hydrocarbons, which are well-known to be characteristic of produced water from both conventional and unconventional formations.³⁴ For other chemicals, the likelihood of detection is less clear. For instance, EPA’s list includes several banned pesticides (dieldrin, aldrin, heptachlor, heptachlor epoxide, lindane, and others) that have been detected in flowback/produced water from hydraulically fractured wells in the Marcellus shale, but do not have reported use in hydraulic fracturing fluids.^{12,34} It is possible that these banned substances were present as residues in the supply water that was used for well injection, or were mobilized from the soil or underground at the well site. More discussion on EPA’s chemical list can be found in our concurrent study.²³

Overall, the chemicals used in hydraulic fracturing fluids or detected in flowback/produced water will vary from site to site, and will depend upon the chemicals that are injected into the well, along with site-specific geological and chemical characteristics of the well formation. To develop a site-specific characterization of potential human health hazards, the toxicity estimates presented here are best used in combination with data on chemicals present at a specific field site.

Qualitative Confidence Analysis. Figure 1 shows all 18 possible permutations of the qualitative confidence score, represented as a decision tree. Based on these possible permutations, 481 (93%) out of the total 515 chemicals received a high confidence score, 29 chemicals (6%) received a medium confidence score, and 5 chemicals (1%) received a low confidence score. The chemicals receiving high confidence scores included 422 chemicals that are used in hydraulic fracturing fluids, and 81 chemicals that have been reported in flowback and produced water.

Figure 1 illustrates how, to have high confidence in a TOPKAT LOAEL estimate, the chemical must be within the chemical space of the model, or at least marginally within it (OPS score of 1 or 2); and have at most 1 unknown fragment (UFS score of 1 or 2). If a chemical is only marginally within the chemical space of the model (OPS score of 1), it must not have any unknown fragments, and must have an LD₅₀ estimate

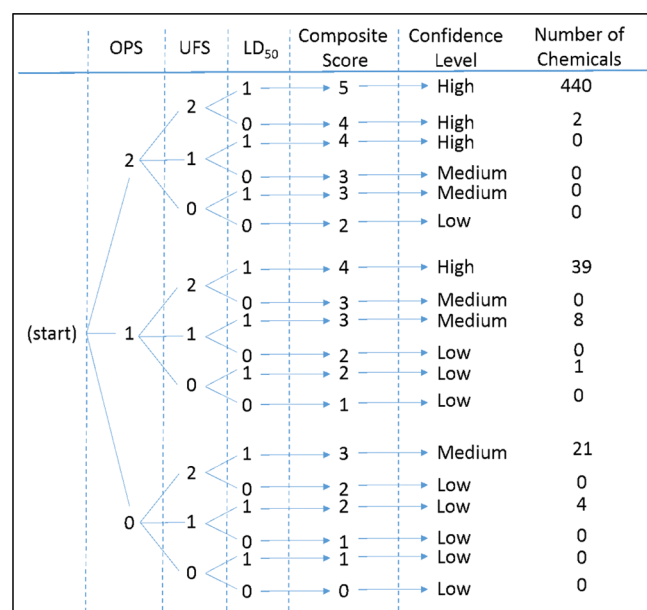


Figure 1. Decision tree to determine confidence in the TOPKAT LOAEL estimates, depicting all possible permutations of the Optimum Prediction Space score (OPS), the Unknown Fragments score (UFS), and the LD₅₀ score (LD₅₀). Starting at the root node, a user follows the line to the OPS, then to the UFS, and finally the LD₅₀ score. The composite score is the sum of these scores, and was used to determine the qualitative confidence level (high, medium, or low). The number of chemicals that received each permutation of the score is shown in the far right column.

greater than the LOAEL estimate, for the LOAEL estimate to gain a rating of high confidence.

Although the relationship between the TOPKAT LOAEL estimate and TOPKAT LD₅₀ estimate is considered as part of the weight of evidence used to calculate the qualitative confidence score, we regard it as being a less reliable metric, since the TOPKAT LD₅₀ model is susceptible to error and limitations. If a chemical is within the OPS of the LOAEL model and has no unknown fragments, a discrepancy in the relationship between the LOAEL and LD₅₀ estimates may simply indicate that the LOAEL model is more accurate than the LD₅₀ model for that chemical. Therefore, we do not feel that this piece of evidence alone is sufficient to lower the prioritization of these chemicals. Out of the 515 chemicals where TOPKAT values were estimated, only two chemicals (triethyl phosphate and phorate) had an LD₅₀ estimate less than the LOAEL estimate. Both of these chemicals had OPS and UFS scores of 2, and therefore received high confidence scores.

QSAR-Based Rank Verification. We next examined the relationship between high-confidence TOPKAT LOAEL estimates and IRIS chronic oral RfDs for chemicals that had both of these values available. IRIS chronic oral RfDs (and other RfVs) are commonly considered in risk management decisions, and risk assessors and risk managers are accustomed to using these values to rank and prioritize chemicals. Thus, it is useful to ascertain how well TOPKAT LOAEL estimates reproduce the ranking of chemicals based on IRIS chronic oral RfDs. We did not attempt to directly compare the TOPKAT LOAEL estimated values to IRIS chronic oral RfD values, since there are likely significant differences between the two values due to several factors: e.g. the application of uncertainty factors

during RfD derivation, as well as the fact that RfDs may be developed from species other than rat and values other than LOAELs.³²

Of the total 481 chemicals in hydraulic fracturing fluids or flowback/produced water with high-confidence TOPKAT estimates, we identified 51 chemicals that also had an IRIS chronic oral RfD (Table 1). Of these 51 chemicals, 3 had IRIS chronic oral RfDs that were derived from a rat LOAEL (see Table 1), and therefore were excluded from the analysis in order to ensure that we did not have any potential overlap with the TOPKAT LOAEL training set. For the remaining 48 chemicals, the IRIS chronic oral RfDs and TOPKAT LOAEL estimates exhibited a Spearman rank correlation of 68% (Figure 2). Permutation testing found a p-value of 1×10^{-11} (Figure 3), indicating that there is a less than 1×10^{-11} chance of a 68% correlation level or larger occurring due to chance. This level of rank correlation indicates that, although there are some differences in rank ordering between the two values, there are many more instances where the ranks were conserved. Therefore, this level of rank correlation provides support for using the TOPKAT LOAEL estimates for the screening and prioritization for further analysis of chemicals that lack an experimentally derived toxicity value.

Although RfDs derived from rat LOAELs were not considered in the Spearman correlation, it is nevertheless important to consider whether the observed correlation was influenced by overlap between the remaining 48 chemicals and the training set. Of the 48 chemicals considered in the correlation analysis, 21 chemicals were present in the TOPKAT LOAEL training set. For the 27 chemicals that were not in the training set, the TOPKAT LOAEL estimates and IRIS chronic oral RfD exhibited a Spearman rank correlation of 51%, with a permutation p-value of 0.003. This conservative analysis removes all chemicals that have potential for confounding, and provides further evidence that the TOPKAT LOAEL estimates provide a largely similar rank ordering of the chemicals as compared to IRIS chronic oral RfDs.

QSAR-Informed Prioritization of Hydraulic Fracturing-Related Chemicals. Since the above results indicate that TOPKAT LOAEL estimates may provide an effective surrogate for toxicity ranking and chemical prioritization, we used the TOPKAT LOAEL estimates to rank the hydraulic fracturing-related chemicals identified by EPA that did not have a chronic oral RfV or OSF available. The application of TOPKAT LOAEL estimates to fill this data gap is illustrated in Figure 4. Of the total 1026 chemicals in hydraulic fracturing fluids or flowback/produced water that lacked an available chronic oral RfVs or OSFs (Figure 4A), TOPKAT LOAEL estimates were available for 417. Of these 417, 389 were rated as high-confidence estimates, while the remaining 28 were rated as medium- or low-confidence (Figure 4B). Although TOPKAT LOAEL estimates carry less weight of evidence relative to RfVs or OSFs, they do provide useful information for chemical prioritization. Therefore, taking this information together, we are now able to rank the potential toxicity of 536 (46%) of the 1173 hydraulic fracturing-related chemicals on EPA's list: 147 chemicals with chronic oral RfVs or OSFs, and 389 chemicals with high-confidence TOPKAT LOAEL estimates (Figure 4C). This is a 3.6-fold increase in the number of ranked chemicals.

This can be further broken down to address data availability for chemicals used in hydraulic fracturing fluids versus chemicals reported in flowback/produced water:

Table 1. All chemicals with IRIS chronic oral RfDs and TOPKAT LOAEL estimates, ordered from most toxic to least toxic based on IRIS chronic oral RfD

CASRN	chemical name	fracturing fluid (FF) or flowback/produced water (FB) ^a	IRIS				TOPKAT	ranks ^f	
			IRIS chronic oral RfD (mg/kg-day)	IRIS POD (mg/kg- day) ^b	total uncertainty factor ^c	POD type ^d	TOPKAT LOAEL estimate (mg/kg) ^e	IRIS Rank	TOPKAT rank
1024-57-3	heptachlor epoxide	FB	0.000013	0.0125	1000	LEL	0.595	1	2
309-00-2	aldrin ^{h,g}	FB	0.00003	0.025	1000	LOAEL	0.743		
60-57-1	dieldrin	FB	0.00005	0.005	100	NOAEL	0.442	2	1
58-89-9	lindane	FB	0.0003	0.33	1000	NOAEL	23.9	3	5
76-44-8	heptachlor ^h	FB	0.0005	0.15	300	NOEL	0.927	4.5	3
107-02-8	acrolein ^h	FF FB	0.0005	0.05	100	NOAEL	45.1	4.5	13
110-86-1	pyridine	FB	0.001	1	1000	NOAEL	69.5	6	18
79-06-1	acrylamide ^h	FF	0.002	0.053	30	BMDL- PBPK	28.7	7	6
98-01-1	furfural ^g	FF	0.003	7.9	3000	LOAEL	54.1		
71-43-2	benzene	FF FB	0.004	1.2	300	BMDL	77.6	8.5	20
91-57-6	2-methylnaphthalene ^h	FB	0.004	3.5	1000	BMDL	103	8.5	25
127-18-4	tetrachloroethylene	FB	0.006	6	1000	LOAEL	36.2	10.5	9
75-09-2	dichloromethane	FF FB	0.006	0.19	30	PBPK	39.7	10.5	10
120-82-1	1,2,4-trichlorobenzene	FB	0.01	14.8	1000	NOAEL	44.5	12.5	11
67-66-3	chloroform	FB	0.01	12.9	1000	LOAEL	47.1	12.5	15
75-27-4	bromodichloromethane	FB	0.02	17.9	1000	LOAEL	61.2	16.5	16
91-20-3	naphthalene ^h	FF FB	0.02	71	3000	NOAEL	67.5	16.5	17
124-48-1	chlorodibromomethane	FB	0.02	21.4	1000	NOEL	73.8	16.5	19
75-25-2	bromoform	FB	0.02	17.9	1000	NOEL	84.8	16.5	22
105-67-9	2,4-dimethylphenol ^h	FB	0.02	50	3000	NOAEL	112	16.5	28
117-81-7	di(2-ethylhexyl) phthalate	FF FB	0.02	19	1000	LOAEL	4040	16.5	48
122-39-4	diphenylamine ^h	FB	0.025	2.5	100	NOEL	30.8	20	7
542-75-6	1,3-dichloropropene	FF	0.03	3.4	100	BMDL	16	22	4
129-00-0	pyrene ^h	FB	0.03	75	3000	NOAEL	36.1	22	8
123-91-1	1,4-dioxane	FF FB	0.03	9.6	300	NOAEL	207	22	38
206-44-0	fluoranthene ^h	FB	0.04	125	3000	NOAEL	44.6	24.5	12
86-73-7	fluorene ^h	FB	0.04	125	3000	NOAEL	95.1	24.5	23
108-39-4	<i>m</i> -cresol	FB	0.05	50	1000	NOAEL	123	26.5	31
95-48-7	<i>o</i> -cresol	FB	0.05	50	1000	NOAEL	229	26.5	40
80-05-7	bisphenol A ^{h,g}	FF	0.05	50	1000	LOAEL	101		
108-88-3	toluene	FF FB	0.08	238	3000	BMDL	163	28	36
71-36-3	1-butanol ^h	FF	0.1	125	1000	NOAEL	117	32	29
75-15-0	carbon disulfide ^h	FB	0.1	11	100	NOEL	126	32	32
100-41-4	ethylbenzene	FF FB	0.1	97.1	1000	NOEL	226	32	39
98-82-8	cumene	FF FB	0.1	110	1000	NOAEL	246	32	41
98-86-2	acetophenone	FF FB	0.1	423	3000	NOAEL	274	32	43
111-76-2	2-butoxyethanol ^h	FF	0.1	1.4	10	BMDL- PBPK	707	32	45
84-74-2	dibutyl phthalate	FB	0.1	125	1000	NOAEL	2090	32	47
100-42-5	styrene ^h	FF	0.2	200	1000	NOAEL	95.7	36.5	24
1330-20-7	xylenes	FF FB	0.2	179	1000	NOAEL	110	36.5	26
78-83-1	2-methyl-1-propanol	FF	0.3	316	1000	NOEL	111	38.5	27
108-95-2	phenol	FF FB	0.3	93	300	BMDL	134	38.5	35
79-10-7	acrylic acid ^h	FF	0.5	53	100	NOAEL	46.8	40	14
78-93-3	methyl ethyl ketone ^h	FB	0.6	594	1000	NOAEL	83.2	41	21
84-66-2	diethyl phthalate ^h	FB	0.8	750	1000	NOAEL	1000	42	46
67-64-1	acetone ^h	FF FB	0.9	900	1000	NOAEL	119	43.5	30
141-78-6	ethyl acetate	FF	0.9	900	1000	NOEL	129	43.5	33
107-21-1	ethylene glycol ^h	FF FB	2	200	100	NOEL	130	45.5	34
85-44-9	phthalic anhydride	FF	2	1562	1000	LOAEL	254	45.5	42
65-85-0	benzoic acid ^h	FF	4	4.4	1	NOAEL	436	47	44
108-94-1	cyclohexanone ^h	FF	5	462	100	NOAEL	191	48	37

^aIndicates whether a chemical was reported in hydraulic fracturing fluids (FF), flowback/produced water (FB), or both (FF FB). ^bPoint of departure (POD) used as the basis for the IRIS chronic oral RfD. ^cTotal uncertainty factor applied to the POD in order to calculate the IRIS chronic oral RfD.

^dLowest observed adverse effect level (LOAEL); lowest effect level (LEL); no observed adverse effect level (NOAEL); no observed effect level

Table 1. continued

(NOEL); benchmark dose lower confidence limit (BMDL); physiologically based pharmacokinetic (PBPK) modeling. ^eTOPKAT LOAEL estimates are rounded to three significant figures. ^fToxicity rankings based on IRIS chronic oral RfDs and on TOPKAT LOAEL estimates. These are the values used in the Spearman rank correlation. In the case of a tie between two chemicals, the average ranking was used. ^gIndicates chemicals with IRIS chronic oral RfDs that were derived from rat LOAELs. These chemicals were excluded from the Spearman rank correlation analysis and were not assigned a rank on this table. ^hIndicates chemicals that were present in the TOPKAT LOAEL model training set.

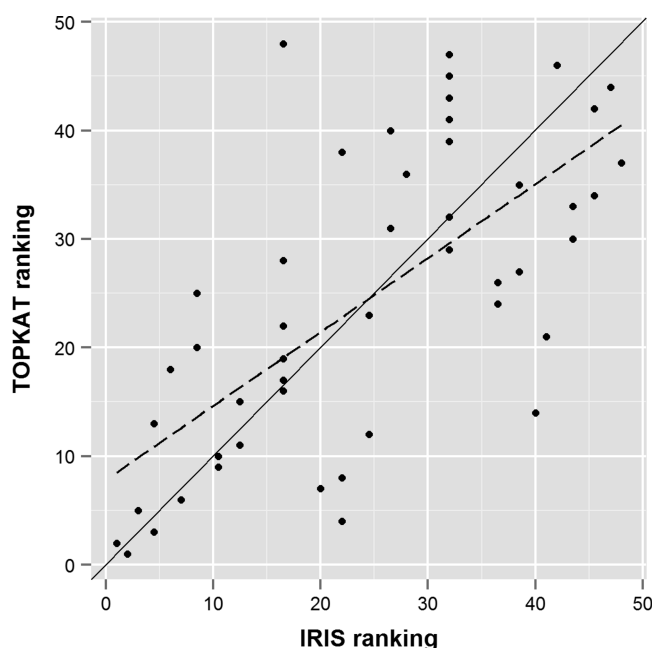


Figure 2. Plot of the Spearman rank correlation results, showing the chemical ranks by TOPKAT LOAEL estimates and by IRIS chronic oral RfDs. Each point represents an individual chemical. The solid line, provided for reference, indicates a correlation of 100%. The dashed line is the best fit through the data, with a Spearman rank correlation of 68%.

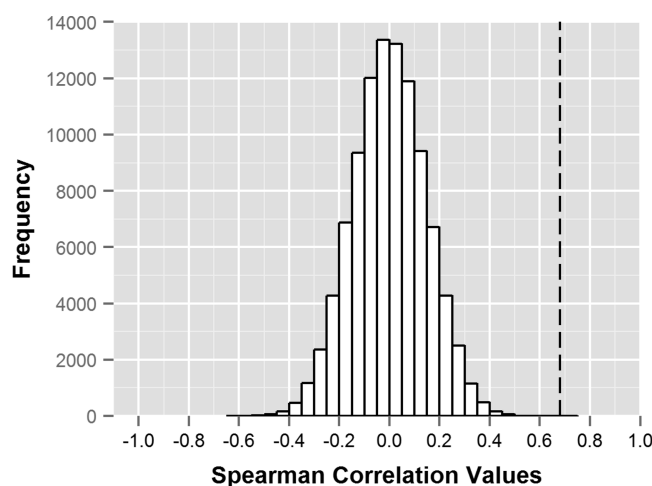


Figure 3. Distribution of random Spearman correlations based on permutation testing, performed using 100 000 iterations. The dashed line, provided for reference, indicates the where the observed Spearman correlation value (0.68 or 68%) fell within the distribution of permutation values.

- High-confidence TOPKAT LOAEL estimates were available for 368 chemicals used in hydraulic fracturing fluids that lack chronic oral RfVs or OSFs. Taken together with the 90 chemicals that have chronic oral

RfVs or OSFs, we are now able to rank the potential toxicity of 458 (43%) of the 1076 chemicals used in hydraulic fracturing fluids. This is a 5.1-fold increase in the number of ranked chemicals.

- High-confidence TOPKAT LOAEL estimates were available for 27 chemicals reported in flowback/produced water that lack chronic oral RfVs or OSFs. Taken together with the 83 chemicals that have chronic oral RfVs or OSFs, we are now able to rank the potential toxicity of 110 (82%) of the 134 chemicals reported in flowback/produced water. This is a 1.3-fold increase in the number of ranked chemicals.

We further honed our analysis by focusing on the chemicals reported in FracFocus 1.0 in at least 10% of disclosures nationwide (Table 2). These chemicals are of particular interest, because they are used frequently and are therefore more likely to be present at hydraulic fracturing sites. As shown in Table 2 and discussed in our concurrent study,²³ 8 (22%) of these 36 chemicals have chronic oral RfVs available, and none have OSFs. Of the 28 chemicals in Table 2 that lack a chronic oral RfV or OSF, we found that 12 have TOPKAT LOAEL estimates available, all of which are high-confidence estimates. Taking this information together, we are now able to rank the potential toxicity of 20 (56%) of these frequently used chemicals: 8 chemicals with chronic oral RfVs or OSFs, and 12 chemicals with high-confidence TOPKAT LOAEL estimates. This is a 2.5-fold increase in the number of ranked chemicals. Of the chemicals that lack chronic oral RfVs and OSFs, methanamine (reported in ~14% of FracFocus 1.0 disclosures) has the lowest TOPKAT LOAEL estimate (12.3 mg/kg). Choline chloride (reported in ~15% of FracFocus 1.0 disclosures) has the next lowest TOPKAT LOAEL estimate (20.8 mg/kg).

Limitations and Uncertainty. There are limitations and uncertainty with respect to the TOPKAT analysis. Although the 68% and 51% Spearman rank correlations are quite good and statistically significant, these results indicate that some chemicals will not be in the proper rank order based on potential hazard. One factor contributing uncertainty to the comparison of these two values is the use of uncertainty factors to derive IRIS chronic oral RfDs. As shown in Table 1, uncertainty factors used in RfD derivation ranged from 1 (i.e., no uncertainty factor was applied) to as high as 3000. In addition, TOPKAT LOAEL estimates are estimates of toxicity in rat chronic oral LOAEL studies, which may not always be appropriate for evaluating human health hazards. It is possible that the rat may not always be the most sensitive species to a given chemical, or that humans may be more or less sensitive than rats for a given biological endpoint. Thus, there is uncertainty as to how well the prioritization will reflect human health hazards.

QSAR models are expected to have a degree of uncertainty, stemming from underlying limitations in the model design. For TOPKAT, one potential problem is that the LOAEL model training set appears to have been last updated in 1995,²⁸ so the

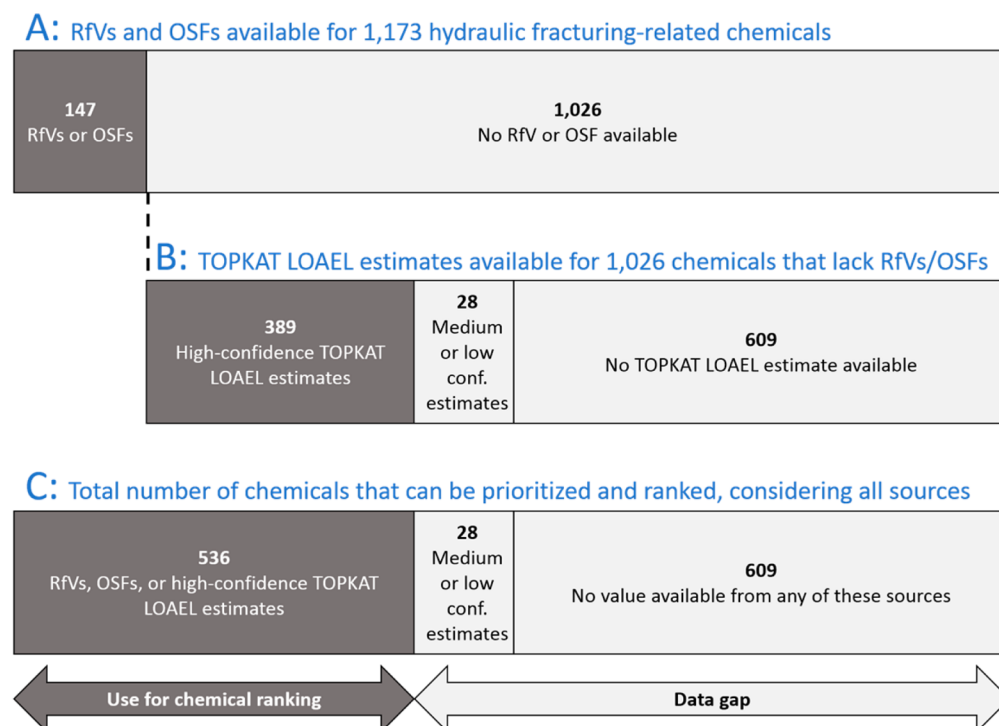


Figure 4. Visual representation of the application of TOPKAT LOAEL estimates to fill data gaps related to the toxicity of 1173 chemicals identified in EPA's hydraulic fracturing study, including chemicals associated with hydraulic fracturing fluids, flowback, and produced water. Dark shading indicates data that can be used to inform chemical ranking and prioritization. Part A depicts the number of chronic oral RfVs and OSFs that are available for these chemicals, identified by EPA as described in the text. Part B depicts the number of high-, medium-, and low-confidence TOPKAT LOAEL estimates that are available for the 1026 chemicals that did not have chronic oral RfVs or OSFs. Part C depicts the total numbers of chemicals that can be ranked and prioritized for further assessment based on toxicity, if TOPKAT LOAEL estimates are used to supplement the available chronic oral RfVs and OSFs.

results do not incorporate toxicity information that has been generated since that time. Additionally, observations by Rupp et al.²⁷ and Venkatapathy et al.²⁸ indicate that the TOPKAT LOAEL model performs better for chemicals that are included in the training set compared to chemicals outside the training set, suggesting that TOPKAT may be overfitting the data. For a list of 347 industrial chemicals that were not included in the training set and met criteria similar to those considered in our qualitative confidence framework (e.g., were within the OPS of the TOPKAT LOAEL model, with no unknown fragments or other warnings), Rupp et al.²⁷ found that between 84 and 99% were predicted within a factor of 100 of LOAELs derived from experimental data. Thus, although these limitations are not ideal, we have knowledge of the uncertainties associated with TOPKAT LOAEL estimates. We have incorporated this information into our analysis by assigning higher confidence scores to chemicals that are similar to the training set by way of the OPS and UFS scores.

One can also evaluate the TOPKAT LOAEL model using the principles defined by the Organisation for Economic Co-operation and Development's (OECD's) *Guidance Document on the Validation of (Quantitative) Structure–Activity Relationship [(Q)SAR] Models*. The OECD principles were developed to provide guidance on the regulatory application of QSAR modeling, and are not intended to act as criteria for regulatory acceptance of QSAR models; however, they are informative. The OECD Principles state that QSAR models should ideally have the following attributes: (1) a defined endpoint; (2) an unambiguous algorithm; (3) a defined applicability domain; (4) appropriate measures of goodness-of-fit, robustness, and

predictivity; and (5) a mechanistic interpretation, if possible.³¹ The TOPKAT models and software do not meet the guidelines specified in Principles 1, 2, or 4. TOPKAT is a “black box” that does not provide a defined endpoint for LOAEL predictions, does not disclose the algorithms used in the model, and does not provide information on internal measures of performance accuracy. These limitations, however, are not uncommon. Many commercial QSAR software programs have proprietary data and software, and operate as “black boxes”. As stated earlier, TOPKAT appears to be the only commercially available software program capable of estimating a quantitative value for a chronic oral LOAEL, and the only program where the LOAEL estimates have been independently evaluated with results published in a peer reviewed journal.

Using QSAR to Bridge Data Gaps for Risk Assessment.

In this study, we have described a novel scientifically based approach for applying TOPKAT LOAEL estimates to facilitate the ranking and prioritization of potentially hazardous chemicals that are associated with hydraulic fracturing activity. We developed a framework for assigning qualitative confidence scores to the estimates, and verified the ability of high-confidence TOPKAT LOAEL estimates to rank the chemicals based on relative hazard. The correlation between TOPKAT LOAEL estimates and IRIS chronic oral RfDs is a highly relevant finding, as IRIS chronic oral RfDs are among the most widely used and highest quality values available for risk assessment. Although previous studies have investigated the relationship between TOPKAT LOAEL estimates and experimentally derived LOAELs, ours is the first study to our knowledge to investigate the correlation between TOPKAT

Table 2. Chemicals Reported in at Least 10% of Disclosures in EPA's Analysis of FracFocus 1.0 (Identified Originally in Yost et al.²³), with Chronic Oral RfVs, TOPKAT LOAEL Estimates, and the Qualitative Confidence Scores for the TOPKAT LOAEL Estimates Provided When Available^{a,b}

CASRN	Chemical Name	Percent of Disclosures	RfV		TOPKAT	
			RfD (mg/kg-day)	Source of RfD	TOPKAT LOAEL estimate(mg/kg) ^c	Qualitative confidence score
14808–60–7	quartz-alpha (SiO ₂)	86.09%	—	—	—	—
67–56–1	methanol	73.10%	2	IRIS	49	medium
64742–47–8	distillates, petroleum, hydrotreated light	67.26%	—	—	—	—
7647–01–0	hydrochloric acid	65.76%	—	—	—	—
107–21–1	ethylene glycol	46.82%	2	IRIS	130	high
67–63–0	isopropanol	46.45%	—	—	81.4	high
7727–54–0	diammonium peroxydisulfate	44.09%	—	—	—	—
9000–30–0	guar gum	39.42%	—	—	—	—
1310–73–2	sodium hydroxide	39.26%	—	—	—	—
107–19–7	propargyl alcohol	33.38%	0.002	IRIS	34.1	low
111–30–8	glutaraldehyde	32.97%	—	—	398	high
7647–14–5	sodium chloride	32.04%	—	—	—	—
64–17–5	ethanol	30.78%	—	—	59.2	high
1310–58–3	potassium hydroxide	30.64%	—	—	—	—
64–19–7	acetic acid	24.63%	—	—	183	high
77–92–9	citric acid	23.93%	—	—	55.8	high
111–76–2	2-butoxyethanol	23.20%	0.1	IRIS	707	high
64742–94–5	solvent naphtha, petroleum, heavy arom.	20.89%	—	—	—	—
91–20–3	naphthalene	18.93%	0.02	IRIS	67.5	high
10222–01–2	2,2-dibromo-3-nitropropionamide	16.46%	—	—	52.4	high
67–48–1	choline chloride	14.83%	—	—	20.8	high
9003–35–4	phenol-formaldehyde resin	14.46%	—	—	—	—
584–08–7	carbonic acid, dipotassium salt	13.93%	—	—	137	high
100–97–0	methenamine	13.72%	—	—	12.3	high
68527–49–1	thiourea, polymer with formaldehyde and 1-phenylethanone	13.23%	—	—	—	—
95–63–6	1,2,4-trimethylbenzene	12.90%	—	—	91.5	high
25322–68–3	polyethylene glycol	12.66%	—	—	—	—
9016–45–9	polyethylene glycol nonylphenyl ether	12.61%	—	—	—	—
68424–85–1	quatarnary ammonium compounds, benzyl-C12–16-alkyldimethyl, chlorides	12.48%	0.44	HHBP	—	—
127087–87–0	poly(oxy-1,2-ethanediyl)-nonylphenyl-hydroxy branched	11.87%	—	—	—	—
12125–02–9	ammonium chloride	11.60%	—	—	—	—
64–18–6	formic acid	11.44%	0.9	PPRTV	125	medium
55566–30–8	tetrakis(hydroxymethyl)phosphonium sulfate	11.42%	—	—	148	high
7758–19–2	sodium chlorite	11.00%	0.03	IRIS	—	—
68439–51–0	alcohols, C12–14, ethoxylated propoxylated	10.63%	—	—	1450	high
7775–27–1	sodium persulfate	10.30%	—	—	—	—

^a— indicates that no value was available. ^bOSFs were not available for any of the chemicals in this table. ^cTOPKAT LOAEL estimates are rounded to three significant figures.

LOAEL estimates and RfDs. Using high-confidence TOPKAT LOAEL estimates as surrogates, we were able to rank a significant number of chemicals that were identified as lacking chronic oral RfVs and OSFs in EPA's hydraulic fracturing study (Figure 4 and Table 2). The increase in the number of ranked chemicals was particularly great for chemicals used in hydraulic fracturing fluids, given that only a small proportion (8%) of those chemicals have chronic oral RfVs or OSFs available from EPA-identified sources.

Using this method, risk assessors and researchers can develop a rank ordering of chemicals at a site based on potential toxicity. Estimated toxicity may be coupled with exposure information, for example, frequency of chemical use, amount of chemical used, or estimates of potential exposure due to environmental fate and transport processes—to create an overall risk ranking

and assist with public health decision making. Researchers may choose to prioritize the monitoring of chemicals with high potential risk, or may initiate studies in order to better understand the toxicity of these chemicals. Such applications have been discussed in other studies.^{35–37} In general, TOPKAT LOAEL estimates can be used to support decisions as to which chemicals at a field site are in most need of further assessment, and help determine which chemicals to assess next. This approach should be used in combination with site-specific data on the chemicals present in hydraulic fracturing fluid, flowback, or produced water.

When it comes to selecting toxicity data for use in risk assessment, it is important to keep in mind that toxicity values exist on a continuum with regards to quality and reliability. The chronic oral RfVs and OSFs identified by EPA are high quality

and reliable toxicity values, which are empirically based and peer reviewed.²³ Other potential sources of toxicity values include state, national, international, private, and academic organizations that did not meet the criteria for inclusion in EPA's hydraulic fracturing study, but could still be high quality sources. Relevant toxicity information may also be found in the scientific literature, including results from guideline studies, high throughput screening assays, and alternative assays. The quality, relevance, or availability of toxicity information from such data sources is not evaluated here. However, it is clear that QSAR-based estimates are one of many possible resources that may be used by risk assessors for the prioritization and analysis of these chemicals.

Although QSAR-based toxicity estimates, specifically the TOPKAT LOAEL estimates, may be lower on the continuum of quality and reliability compared to experimentally derived toxicity values, the approach we describe here has certain advantages as a complementary analysis. Chief among them, QSAR models require only a chemical structure to estimate a toxicity value, and provides a rapid means of filling data gaps related to chemical toxicity. The methods we present assign qualitative confidence scores to the TOPKAT LOAEL estimates, and address some of the uncertainties and limitations associated with these estimates. When faced with numerous data-poor chemicals at a field site, risk assessors and researchers may find these methods useful in their effort to identify potential hazards.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b05327.

TOPKAT LOAEL estimates for 515 chemicals, and a TOPKAT LOAEL-based rank ordering of 417 chemicals that lacked chronic oral RfVs or OSFs from EPA's hydraulic fracturing study (XLSX)

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Notes

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