

Forensic analysis of *tertiary*-butyl alcohol (TBA) detections in a hydrocarbon-rich groundwater basin

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Abstract *Tertiary*-butyl alcohol (TBA), a high-production volume (HPV) chemical, was sporadically detected in groundwater and coalbed methane (CBM) wells in southeastern Colorado's hydrocarbon-rich Raton Basin. TBA concentrations in shallow water wells averaged 75.1 µg/L, while detections in deeper CBM wells averaged 14.4 µg/L. The detection of TBA prompted a forensic investigation to try to identify potential sources. Historic and recent data were reviewed to determine if there was a discernable pattern of TBA occurrence. Supplemental samples from domestic water wells, monitor wells, CBM wells, surface waters, and hydraulic fracturing (HF) fluids were analyzed for TBA in conjunction with methyl *tertiary*-butyl ether (MTBE) and ethyl *tertiary*-butyl ether (ETBE), proxies for evidence of contamination from reformulated gasoline or associated oxygenates. Exploratory microbiological sampling was conducted to determine if methanotrophic

organisms co-occurred with TBA in individual wells. Meaningful comparisons of historic TBA data were limited due to widely varying reporting limits. Mapping of TBA occurrence did not reveal any spatial patterns or physical associations with CBM operations or contamination plumes. Additionally, TBA was not detected in HF fluids or surface water samples. Given the widespread use of TBA in industrial and consumer products, including water well completion materials, it is likely that multiple diffuse sources exist. Exploratory data on stable isotopes, dissolved gases, and microbial profiling provide preliminary evidence that methanotrophic activity may be producing TBA from naturally occurring isobutane. Reported TBA concentrations were significantly below a conservative risk-based drinking water screening level of 8000 µg/L derived from animal toxicity data.

Keywords *Tertiary*-butyl alcohol · Coalbed methane · Hydraulic fracturing · Groundwater · Risk-based screening levels

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Introduction

Natural gas that is associated with organic-rich sedimentary materials is typically produced through a combination of thermogenic and microbiological processes. Hydrogeologic conditions affect whether the thermogenic or biogenic gas remains in place or migrates to shallower formations. For example, the gas trapped in coals may remain in place until the water pressure is

reduced, or migrate along preferential flow paths such as fractures or dykes. Advances in hydraulic fracturing (HF) in recent decades have made extraction of coalbed methane (CBM) gas economically feasible. The focus of this paper is the Raton Basin (Jurich and Adams 1984; Johnson and Finn 2001), a hydrocarbon-rich 5700-km² asymmetrical trough extending from southern Colorado into northern New Mexico that contains significant coal resources (Gromer 1982; Johnson and Finn 2001), several major surficial geologic features, structural features, and historic coal mines that were active from the 1800s to mid 1990s (Gromer 1982; Jurich and Adams 1984; Geldon 1989; Stevens et al. 1992; Hemborg 1998; Johnson and Finn 2001; Lewicki 2001; Worrall 2004). The use of HF within the 3100-km² Colorado portion of the Raton Basin began in the 1990s, and currently, there are over 4000 CBM wells. Raton Basin CBM wells are primarily completed in and produce gas from the deeper portion of the Raton and Vermejo formation coal seams which contain economic quantities of CBM gas. The abundant CBM gas tends to be methane-dominated with lesser amounts of higher-chain hydrocarbons, including ethane, propane, butane, isobutane, and its prevalence across the basin (e.g., within the Poison Canyon, Raton and Vermejo formations) predates CBM development (Gromer 1982; Jurich and Adams 1984; Johnson and Finn 2001). In addition to the development of CBM wells in the basin, over 6500 domestic water wells in the Raton Basin are relied upon by the rural residents for water supply. The domestic water wells are typically drilled and completed in shallow alluvium or into bedrock of the Poison Canyon or Raton formations. Typically, the domestic water wells are open-hole completions only having surface casing grouted to 12 m or less and occasionally completed in or through shallow coal seams. The Raton Basin was one of six retrospective case studies that the US Environmental Protection Agency (EPA) selected to investigate potential effects of HF on potable groundwater (EPA 2011, 2015a).

In parallel with hydraulic fracturing and CBM development, groundwater monitoring programs have been instituted to identify potential impacts on groundwater quality and quantity, particularly when drinking water wells are in close proximity to oil and gas operations (National Research Council 2010, 2014; Dahm et al. 2011; Colorado Oil and Gas Conservation Commission (COGCC) 2009, 2012). The COGCC regulates CBM activities and stipulates monitoring requirements in the

State of Colorado, including the Colorado section of the Raton Basin (COGCC 2009, 2012). Targeted monitoring is used to respond to citizen concerns, investigate surface spills, and identify contaminants that might pose potential health risks. The COGCC water quality monitoring program incorporates a suite of inorganic and organic parameters including major ions, trace metals, dissolved gases, fuel-related compounds, stable isotopes of carbon, and hydrogen associated with dissolved methane, and microbiological testing. One chemical that has been sporadically detected in Raton Basin groundwater is *tertiary*-butyl alcohol (TBA) (CASRN 75-65-0) (EPA 2015a; Gintautas 2015), a high-production volume (HPV) chemical that is considered relevant to HF because of its role in production of a gel breaker used by some oil and gas operators (EPA 2012a), *tertiary*-butyl hydroperoxide (TBHP) (CASRN 75-91-2). TBHP can be converted to TBA in natural environments (European Chemicals Agency 2006) and can also contain manufacturing residuals. The objectives of this paper are to present a forensic analysis of TBA occurrence in Raton Basin groundwater, compile biogeochemical evidence for potential sources, and discuss the potential human health implications of the observed concentrations.

TBA overview

TBA is a high-production volume (HPV) chemical in the USA and Europe, with numerous industrial and chemical uses and a reported aggregated US production and/or import volume of over 0.45 billion kilograms (EPA 2009). In 2012, over 0.68 million kilograms of TBA were reported disposed or otherwise released by 11 industrial categories in the USA, with the highest releases associated with hazardous water/solvent recovery (North American Industry Classification System (NAICS) 562) and chemicals (NAICS 325) (EPA 2012b). First used in US gasoline as an octane booster when tetraethyl lead was being phased out in the 1970s, TBA is best known as a production intermediate and breakdown product of the fuel oxygenates methyl *tertiary*-butyl ether (MTBE) and ethyl *tertiary*-butyl ether (ETBE) (California Environmental Protection Agency 1998; Clark 2002; Interstate Technology and Regulatory Council 2005; HSDB 2014) and the octane booster *tertiary*-butyl acetate (TBAC) (Richardson et al. 1961). However, more relevant to widespread human

exposure are its many uses in commercial products: in nitrocellulose lacquers and latex production; as a solvent in paint removers and lacquers; and as an alcohol denaturant that can comprise up to 5 % of numerous cosmetics, fragrances, deodorants, hair and skin care products, automotive products, and household cleaning products (National Library of Medicine 2014).

TBA is used as a direct food flavoring ingredient and as a fruit essence (National Toxicology Program (NTP) 1995; Clark 2002; Chen 2005; HSDB 2014; National Library of Medicine 2014), and is a major ingredient of fusel oil, a byproduct of the fermentation or distillation process in the production of alcoholic beverages (Chen 2005). It is permitted by the US Food and Drug Administration as an indirect food additive, and may be used as a defoaming agent for commercial products used in “producing, manufacturing, packaging, processing, preparing, treating, transporting, or holding food” (21 CFR 176.200), and also as a surface lubricant for metallic utensils or surfaces that contact food (21 CFR 178.3910). TBA is classified as a class 3 chemical of low toxicity under US pharmacopeia regulations, which permit consumption of TBA in pharmaceuticals at levels of up to 50 mg per day (Sullivan et al. 2010).

From a biogeochemical perspective, TBA is a water-soluble branched-chain alcohol with a camphor-like odor and limited volatility, organic carbon sorption, environmental persistence, or bioaccumulation potential (Hazardous Substances Data Bank (HSDB) 2014). Based on its physicochemical characteristics, TBA is most likely to distribute to water, where it is highly mobile (Arp et al. 2005; HSDB 2014). Microbial degradation of TBA has been reported to occur under anaerobic (Finneran and Lovley 2001; Wei and Finneran 2011) or aerobic conditions (Bradley et al. 1999; North et al. 2012), in surface water and sediments (Bradley et al. 1999, 2002), and in subsurface environments (Fayolle et al. 2001; Schmidt et al. 2004; Eweis et al. 2007; Greenwood et al. 2007; Landmeyer et al. 2010; Rasa et al. 2011). Both aerobic and anaerobic microbial isobutane metabolism can generate TBA (Hou et al. 1982; Jones et al. 2007). While data on the prevalence of isobutane in groundwater are limited, it is commonly associated with petroleum/natural gas deposits (Forrest et al. 2011), and intermittent entrainment of air during drilling, pumping, or groundwater recharge can support aerobic microbial activity. The ability of some alkane-oxidizing microorganisms, especially methane-oxidizing bacteria (methanotrophs), to

cometabolically oxidize isobutane to TBA with high efficiency under aerobic conditions has long been recognized (Hou et al. 1982; Jones et al. 2007). Methanotrophs are able to oxidize methane under either aerobic or anaerobic conditions and are also able to oxidize isobutane to TBA (Hou et al. 1982). However, such processes have not been documented in the field.

Given its large-scale production and widespread use in industrial and consumer products, as well as natural production by microbial processes, it is reasonable to expect TBA to be present in the ambient environment. Exposure of the general public is suggested by the fact that TBA concentrations in blood samples ranged from 0.69 to 3.61 $\mu\text{g/L}$, with a median of 1.13 $\mu\text{g/L}$ in the Centers for Disease Control and Prevention’s National Health and Nutrition Examination Survey III Priority Toxicant Reference Range Study (Bonin et al. 1995). Moreover, detectable levels of TBA were documented in blood samples from participants in human MTBE metabolism studies (as well as unexposed rats), conducted in the USA and Europe, prior to controlled testing of MTBE exposure (Amberg et al. 1999; Prah et al. 2004). However, because TBA has not been regularly monitored in environmental media or biological specimens, the extent of human exposure is unquantified. Historically, the primary focus of TBA monitoring has been associated with groundwater and surface water investigations relevant to known or suspected reformulated gasoline fuel spills (Deeb et al. 2003; Shih et al. 2004; Wilson et al. 2005; Rosell et al. 2006; Hopple et al. 2009; Carter et al. 2012; Bowers and Smith 2014). In the 1970s, TBA was detected in some US drinking water sources (Dowty et al. 1975; Coleman et al. 1976; Lucas 1985) and, as such it was included in the National Academy of Sciences Drinking Water and Health Committee’s initial list of 298 VOCs that were screened for potential health risks (National Research Council 1977). However, it was not ranked as a high-priority waterborne contaminant and not included on any of the subsequent contaminant candidate lists developed by the EPA under the requirements of the Safe Drinking Water Act amendments of 1996 (P.L. 104-182). In addition to consumption of TBA in food and drinking water, inhalation of air containing TBA (and/or its metabolic precursors) is a likely human exposure route. TBA has been detected in ambient air samples from semi-rural to urban locations throughout the USA (Pankow et al. 2003), but it is not classified as a hazardous air pollutant, and is not routinely measured at EPA air toxics monitoring sites.

Neither the EPA nor the International Agency for Research on Cancer (IARC) has yet developed toxicological criteria for TBA. Thus, evaluation of potential human health risks associated with TBA in Raton Basin water wells is complicated by the lack of consensus on risk-based screening levels. Until such criteria are developed, comprehensive toxicological evaluations published in the past two decades by NSF International (NSF International 2003), the American Petroleum Institute (American Petroleum Institute 2005), and the Cosmetic Ingredient Review Panel (Chen 2005) provide the most appropriate basis for evaluating the potential human health risks associated with exposure to TBA through drinking water.

Materials and methods

This study is a forensic analysis of publically available Raton Basin water quality data supplemented by biogeochemical monitoring of shallow (21 to 253 m deep) groundwater wells and deeper (432 to 969 m deep) CBM wells. The shallow domestic water wells sampled are typically open-hole completions with cement grout from surface down to approximately 12 m, while the CBM wells are cemented over hundreds of meters at intervals of shallow domestic wells, and completed in Raton and Vermejo formation coals with the first perforations on average at 424 m below ground surface. Samples were also collected from permitted outfalls or discharge locations that release CBM produced water in compliance with State of Colorado permit requirements. Data from supplemental monitoring of HF fluids were also reviewed, along with results from exploratory bench-scale batch leaching tests of electrical tape and pipe thread sealant used in domestic water well submersible pump installations to determine if detectable quantities of TBA could be mobilized during pumping or routine maintenance activities (Gintautas 2015).

Data sources

Approximately a decade of sampling data collected between 2005 and 2014 were reviewed and assessed, including results from recent sampling efforts (2010 to 2014) that targeted TBA and were conducted by Norwest Corporation (Norwest), the COGCC, and Glibota Environmental Inc. (Glibota). Sampling data were compiled, sorted, and reviewed to identify

sampling locations where TBA had been a laboratory target compound or reported as a tentatively identified compound (TIC). Prior to 2008, TBA was identified as a non-target TIC in two water wells, the first was in 2006. Since 2010, TBA has been a target analyte at over 80 wells across the Raton Basin. Data reported by COGCC were also reviewed. Data reported by EPA (2015a) were not reviewed in this study, but observed trends were compared.

Supplemental sampling, testing, and analysis

Five types of water samples were evaluated: (1) domestic water and groundwater monitoring wells, herein referred to as water wells (21–253 m deep) ($n=44$); (2) CBM wells (432 to 969 m deep) ($n=37$); (3) surface water sites downstream of state-permitted CBM discharges ($n=4$); (4) co-mingled CBM-produced water (from multiple wells) collected from discharge pipes permitted under the State of Colorado National Pollution Discharge Elimination System permits (NPDES) ($n=13$), and (5) HF-related samples (four HF flowback samples and one sample of pre-injection HF fluid).

Water well locations

Of the 46 water wells that were sampled, six are dedicated groundwater-monitoring wells that were installed between 2006 and 2008 at depths similar to local domestic water wells, but with more rigorous and consistent drilling and construction practices. The monitor wells enable evaluation of groundwater quality in shallow aquifers and provide a basis for investigating potential connections between shallow groundwater and CBM well drilling, HF, and production. All samples from water wells that were not in routine use were collected after purging at least three well bore volumes and ensuring that field parameters of temperature, pH and conductivity were stable (<20 % change between measurements). For those water wells in operation and used daily, field parameters were collected and immediately followed by sample collection, with minimal purging.

Each site was sampled one or more times for TBA, with some sites sampled only for TBA, and other sites sampled for additional geochemical parameters including inorganics, stable isotopes, and organic constituents. One of the water wells, designated Water Well A (WWA), underwent more comprehensive multi-year sampling at the request of the owner.

CBM wells and produced water discharge locations

CBM well samples were collected directly from CBM wellheads without purging. Typically, CBM well usage is continuous, although there can be intermittent down time during operational changes or maintenance. Some CBM well samples were collected using pump jacks with pumping rates of about 0.5 l per minute, while other CBM well samples were collected using progressing cavity (PC) pumps which can produce as much as several liters per minute. In addition to CBM well samples, CBM-produced water was sampled at thirteen state-permitted discharge locations that collectively represent approximately 45 % of the total 12 million liters per day of CBM-produced water discharged within the Raton Basin.

Investigation of potential sources of TBA related to hydraulic fracturing activities

The COGCC conducted bench-scale testing of the potential for TBA to leach from two products that are commonly used in domestic wells: green waterproof electrical tape and pipe thread sealant (Gintautas 2015). Aliquots of each product were weighed into 40-ml volatile organic analysis (VOA) vials, immersed in volatile-free blank water, sealed, and stored at ambient temperature for 6 days to mimic groundwater immersion. The mass-to-volume ratios were 167 g/l for the electrical tape and 25.5 g/l for the pipe sealant. Following the 6-day contact period, the VOC content of each vial was analyzed.

Sampling protocols and analytical methods

All samples were collected by trained personnel (Norwest or the COGCC) who followed rigorous quality control protocols that included field blanks, trip blanks, and duplicate samples (approximately one in ten samples). Samples were analyzed by two independent certified laboratories including Test America (Savannah, Georgia) and Energy Laboratories (Billings, Montana). In some cases, duplicate samples were sent to each of the two certified laboratories to enable cross-checking of the data in terms of detection and quantification of TBA. Data assessment included third party quality control review of organic laboratory instrument output by Environmental Standards, Inc. (Rodgers 2014), to confirm identification and quantification of

TBA results and to determine if there were consistent patterns of other low-level organics that could be co-contaminants. Analytical instrument organic data were also reviewed to identify TICs that could be potentially related to HF or TBA.

VOC analytical methods

The majority of VOC analyses were performed by Test America (Savannah, Georgia) using EPA method 524.2 with a standard reporting limit (RL) of 1.6 to 2 µg/l for TBA, Energy Laboratories (Billings, Montana) using EPA method 8260B with a standard RL of 5 µg/l for TBA, and ESC Lab Sciences (Mt. Juliet, Tennessee) using EPA method 8260B with a standard RL of 5 µg/l for TBA. A small number of analyses were performed by ALS Environmental (Ft. Collins, Colorado) on samples collected by the COGCC using method 8260B with TBA RLs ranging from 50 to 200 µg/l. The samples collected for analysis by Test America used hydrochloric acid as a preservative, while Energy Laboratories samples were preserved with trisodium phosphate (TSP). The TSP preservative eliminates the potential for acid hydrolysis of MTBE to TBA in the sample containers if MTBE is present. Reformulated gasoline-related compounds that were also analyzed included benzene, ethylbenzene, toluene, xylenes, n-octane, cyclohexane, and other fuel oxygenates *tert*-amyl alcohol (TAA), *tert*-amyl methyl ether (TAME), ETBE, and MTBE as a target compounds. MTBE was analyzed at an RL of 0.5 and 1 µg/l by Test America and Energy Laboratories, respectively. In addition to these target compounds, TICs were reported by Test America and Energy Laboratories to assess the potential for missed target compounds that may be associated TBA detections.

Dissolved gas and stable isotope analysis

Dissolved gas samples were collected by Norwest and the COGCC using dissolved gas bottles filled underwater in a larger container as per the laboratory recommendations, a modification of the United States Geological Survey (USGS) sampling protocol for dissolved gases (USGS 2013). Benzalkonium chloride (BAK) was added as a biocide to prevent microbial alteration of the sampled gas. Dissolved gas analyses were conducted by Isotech Laboratories, Inc. (Isotech).

Isotech dissolved gas analysis included fixed gases (helium, carbon monoxide, oxygen + argon, nitrogen, and carbon dioxide) and hydrocarbons having from one to more than six carbon atoms (methane, ethane, ethene, propane, isobutane, n-butane, isopentane, n-pentane, and hexane grouped with all alkanes having more than six carbon atoms) reported as mole percent. Isotech dissolved gas analyses were performed using a Shimadzu 2010 Gas Chromatograph (GC) with a thermal conductivity detector for analysis of fixed gases and a flame ionization detector for analysis of hydrocarbons.

Stable carbon isotope analyses of dissolved methane and dissolved carbon dioxide gas were also conducted by Isotech. Stable isotopes of methane were measured only when concentrations were high enough to use isotope ratio mass spectrometry. The lower limit of methane needed for isotope analysis ranged from 0.5 to 1 mg/l, depending on the sample matrix and instrumentation performance. The carbon isotope analysis of methane gas was performed using a SRI 8610C GC preparation system which oxidizes the hydrocarbons into carbon dioxide and water which are purified and then analyzed using a dual inlet Finnigan MAT Delta S isotope ratio mass spectrometer. Similarly, the carbon dioxide isotope ratios of carbon were analyzed with a Finnigan MAT Delta S isotope ratio mass spectrometer.

Microbiological testing

The COGCC and Norwest conducted microbiological sampling on seven water wells and two CBM wells. Methane was detected in all nine wells, while TBA was detected in six of the wells. Microbiological testing was performed by Microbial Insights, Inc. for phylogenetic group testing of methanotrophs using a CENSUS® MOB assay to quantify specific active microorganisms, groups of microorganisms, or functional genes involved in biological processes based on quantitative polymerase chain reaction (qPCR) methods. In particular, the qPCR MOB assay detects Type I (Methylococcaceae family), Type II (Methylocystaceae family), Type X (Methylococcus), and others including Crenothrix and Clonothrix methanotrophs.

In addition to DNA analysis, two samples from one well (WWA) were evaluated for messenger RNA (mRNA) expression of soluble and particulate methanotrophic enzymes: soluble methane

monooxygenase (sMMO) and particulate methane monooxygenase (pMMO). Because DNA is stable in dead cells, DNA results may not reflect active cells. Therefore, mRNA expression is used to differentiate active metabolic processes (Han and Semrau 2004). Methanotrophs use methane monooxygenases (pMMO and sMMO) to catalyze the oxidation of methane to methanol (pMMO is the membrane-bound particulate form, and sMMO is the cytoplasmic soluble form) (Paszczynski et al. 2011). These enzymes are known to have broad substrate specificities allowing for the biotransformation of higher-chain alkanes (e.g., ethane, propane, butane, and pentane) to alcohols via oxidation and co-metabolism (Jiang et al. 2010).

Results and discussion

The widespread presence of TBA in products that are routinely used in day-to-day activities in residential, commercial, and industrial environments makes it difficult to identify specific sources of TBA in the environment. Although HF in the Raton Basin predated the earliest TBA detection, the first reported detection was in 2006 at a domestic water well approximately 1 km from the nearest CBM well. It is also important to note that the reporting limit for historic data was typically 50 µg/l, and it was not possible to verify TBA detections that were reported as a TIC in samples prior to 2008 because laboratory data files are not available. Thus, the apparent lack of detection of TBA in historical samples may be an artifact of high RLs or because it was not a target compound.

TBA detections

Results of TBA sampling are summarized in Table 1 in terms of how many sites were sampled (No. sites sampled), the total number of TBA analyses for each site category (No. analyses), and the number of detection (No. detections). In some cases, individual sites were sampled multiple times as reflected by the number of detections. The number of non-detects having an RL less than or equal to 5 µg/l are also listed to show the complete sample size with respect to TBA analyses where many samples were associated with relatively higher RLs (50 to 200 µg/l) that are not directly comparable to sample analyses

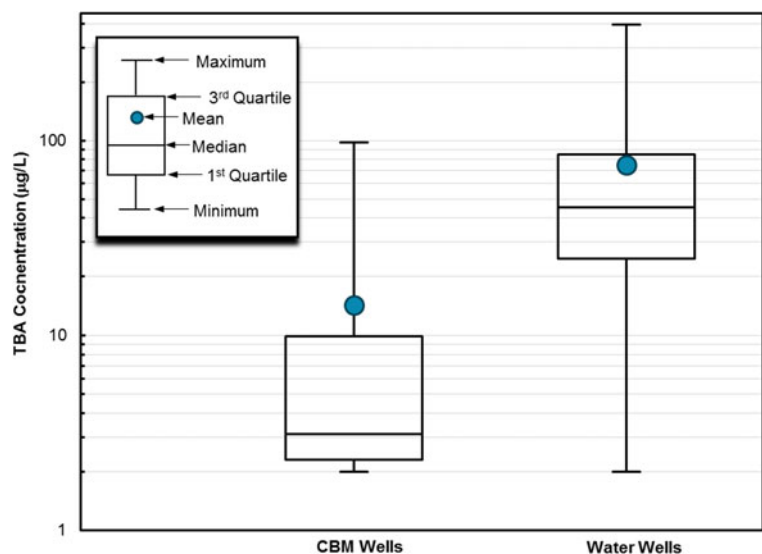
Table 1 Summary of the numbers and types of TBA samples and detections in Raton Basin water samples

Site type	No. sites sampled	No. analyses	No. sites with detections	No. detections	No. non-detect analyses with RL ≤ 5 $\mu\text{g/l}$
Water wells	44	118	14	51	29
CBM wells	37	117	11	27	73
CBM discharge points	13	27	0	0	24
Surface water sites	4	12	0	0	4
<i>Total</i>	<i>98</i>	<i>274</i>	<i>25</i>	<i>78</i>	<i>130</i>

having an order of magnitude lower RL (5 $\mu\text{g/l}$ or less). TBA was detected in approximately 32 % of the 44 water wells and 30 % of the 37 CBM wells that were sampled. However, it was not detected in any of the surface water or CBM-produced water discharge point samples. Assuming stationarity, well samples were averaged for each site having more than one result. The maximum water well and CBM well TBA concentrations are 395 and 98.2 $\mu\text{g/l}$, with averages of 75.1 and 14.4 $\mu\text{g/l}$, respectively (Fig. 1). TBA detections were also reported in the Raton Basin HF retrospective case study conducted by the USEPA (EPA 2015a) with similar trends.

Concentrations of TBA were assessed spatially and in conjunction with proximity to geological structures including mapped faults, dykes and sills, and population centers, to identify potential trends. However, there was no discernable pattern to the occurrence of TBA. In general, the wells where TBA was detected were

frequently separated by several kilometers and interspersed with wells without detectable TBA (Fig. 2). In all cases, TBA detections were associated with wells in rural areas and, in a few cases, mapped geologic structures were nearby. Concentrations of TBA were not related to well depth when analyzed as a whole, but when analyzed as two separate groups, water wells and CBM, the shallower water wells (21 to 253 m deep) had significantly higher concentrations of TBA than deeper CBM wells (432 to 969 m deep) based on a Kolmogorov-Smirnov non-parametric test ($p < 0.001$). Additionally, no correlation was identified between TBA concentrations and water production rates or well usage. TBA was detected at similar concentrations regardless of whether the well was used routinely or infrequently. For the CBM wells, detected levels of TBA were similar, regardless of whether low-flow pump jacks or higher flow PC pumps were used for sample collection.

Fig. 1 Box-and-whisker plot of TBA detections in Raton Basin groundwater samples grouped by well type

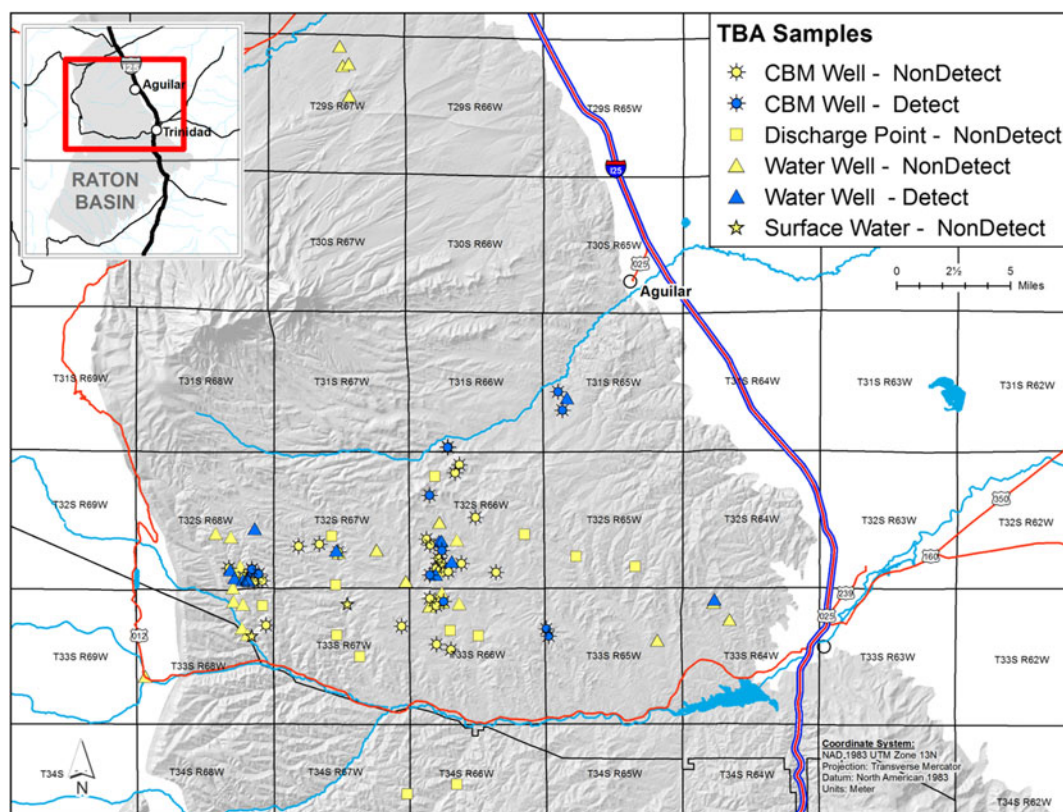


Fig. 2 TBA sampling locations in the central Raton Basin grouped by sample type with detects and non-detects identified

Investigation of Raton Basin groundwater TBA sources

TBA precursors

The reformulated gasoline-related TBA precursors, MTBE and ETBE, were not detected or identified in any of the water wells, CBM well, or produced water samples. The lack of detection of these compounds suggests that the presence of TBA in sampled Raton Basin wells is not attributable to ether oxygenates in reformulated gasoline. HF materials used by Raton Basin's largest CBM operator do not list TBA as an ingredient, nor any chemicals known to degrade to TBA, such as TBHP. TBA was not detected in the samples of HF flowback water ($n=4$, RL=2 to 200 $\mu\text{g/l}$), or HF fluids pre-injection ($n=1$, RL=9.6 mg/kg). Further, no TBA precursors were identified in a review of target or TIC organic analytical data.

The batch leachate testing of electrical tape used in installation of a submersible pump at a single water well was reported as non-detect for TBA, and no peak was observed in instrument output. The TBA concentration

associated with the pipe sealant batch leachate testing after 6 days of immersion in volatile-free water was below the laboratory's established limit of detection (LOD=350 $\mu\text{g/l}$) and reported as non-detectable. However, the TBA peak was clearly identifiable, and estimated from the instrument output at approximately 290 $\mu\text{g/l}$ (Gintautas 2015). The LOD was elevated due to dilution of the sample for higher concentrations of other volatile organics including acetone. In the water well sample collected using the submersible pump and pipe with the pipe thread sealant, TBA was detected at the RL of 2 $\mu\text{g/l}$. This well is completed in bedrock in a rural location, approximately three miles from the closest CBM well. The pipe thread sealant used is a common water well product, but not used with CBM wells, and cannot be traced to individual water wells due to a lack of data on well construction materials. Thus, while these results indicate that the use of standard water well materials in submersible pump installations can result in low-level detections of TBA (and as such exemplify the presence of TBA in products in which it is not a listed ingredient), they do not account for its

presence in CBM wells. TBA release from the pipe thread sealant would be expected to decline with time after the initial application, but concentrations in individual water wells have varied over years of continuous use and large volumes of water produced. Finally, the pipe thread sealant analysis also produced positive detections of acetone, chloroethane, and vinyl acetate, and detections above the method detection limit but below the RL for 2-butanone and toluene. The fact that none of these compounds were identified as co-contaminants in other water samples containing TBA suggests that pipe thread sealant is not a likely source of TBA in all the Raton Basin groundwater wells sampled and cannot account for all of the detections identified across the basin.

Evaluation of evidence for microbial production of TBA

Review of the entire data set coupled with detailed analysis of isotopic and microbiological data from an individual well (WWA) provides three lines of evidence for potential microbial oxidation of isobutane to TBA. First, isobutane, the necessary substrate for microbial production of TBA, was detected in 50 of 370 (13 %) water samples collected from 45 of 161 (28 %) sites (Table 2). The number of analyses is greater than the number of sites sampled, due to multiple sampling events for several of the sites. Isobutane was not always co-detected with TBA.

Isobutane is a common component of natural gas that frequently co-occurs with methane and other hydrocarbons (Forrest et al. 2011). In general, isobutane concentrations tended to be lower in the deeper CBM wells as compared to the shallower water wells, although based on a Kolmogorov-Smirnov non-parametric test the two distributions appear similar ($p > 0.001$). The fact that isobutane was not consistently co-detected with TBA could be due to spatial or temporal differences in microbial activity that affect the relative rates of TBA production along with inherent natural isobutane variability

associated with the relative depth and thermal maturity of the gas deposits.

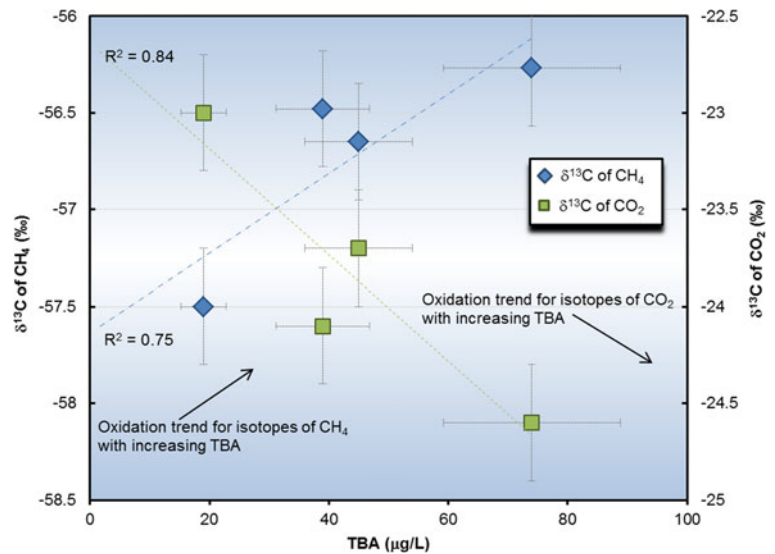
Methane and carbon dioxide carbon stable isotope values ($\delta^{13}\text{C}$) from WWA, while only available for four data points, displayed a strong relationship with TBA concentrations ($R^2 = 0.75$, and 0.84 , respectively), and provide evidence for methane oxidation (Fig. 3). Trends of dissolved methane gas oxidation, identified by the enrichment of ^{13}C (higher $\delta^{13}\text{C}$) in residual methane (Fig. 3), and depleted ^{13}C (lower $\delta^{13}\text{C}$) in residual carbon dioxide (Fig. 3), are consistent with microbial natural gas oxidation. This isotopic evidence of dissolved methane oxidation is consistent with the possibility of microbial TBA production from isobutane oxidation. The biodegradation or oxidation of organic compounds, such as methane and isobutane, can shift the isotopic ratio of reactants and products due to preferential bond cleavage of lighter isotopes in microbial pathways (Clark and Fritz 1997). This shift in isotope ratios, termed kinetic isotope fractionation, occurs because the molecular bonds formed by the heavier isotope (^{13}C) are stronger relative to the lighter isotope (^{12}C). The kinetic fractionation is a result of the lighter isotope (^{12}C) being removed from the parent compound, methane or isobutane in this case, at a faster rate than ^{13}C , causing enrichment of ^{13}C in the residual reactant and enrichment of ^{12}C in the metabolite, carbon dioxide in the case of methane oxidation (Clark and Fritz 1997).

If the available microbial biomass is capable of oxidizing methane, it is also plausible that the conditions are conducive for oxidation of higher-chain hydrocarbons including ethane, ethene, propane, n-butane, and isobutane. Additionally, TBA may be cometabolically produced during aerobic microbial isobutane oxidation by the resident methanotrophic consortia responsible for methane oxidation (Hou et al. 1982; Jones et al. 2007). Even though there is a lack of correlation between isobutane and TBA detections in WWA, the observed patterns likely reflect the net result of biogeochemical

Table 2 Summary of isobutane detections in Raton Basin water samples

Site type	No. sites sampled	No. analyses	No. sites w/ detections	No. detections	Average (mole%)
Water wells	61	237	16	23	0.019
CBM wells	90	113	28	26	0.0045
CBM discharge	1	3	1	1	0.0003
Surface water	9	17	0	0	ND
<i>Total</i>	<i>161</i>	<i>370</i>	<i>45</i>	<i>50</i>	

Fig. 3 Methane and carbon dioxide carbon isotope ratios versus TBA concentrations in Water Well A



oxidation reactions. It is also possible that some of the fluctuations in methane and carbon dioxide isotopic values can be explained by natural variation. No evidence of reformulated gasoline fuel contamination or other anthropogenic contamination was observed in WWA that could serve as a source of TBA, and methane was the only organic analyte consistently co-detected with TBA. A time-series comparison of methane and TBA concentrations in WWA (Fig. 4) reveals a consistent co-variance of these parameters, with a correlation coefficient (R^2) of 0.35. Although oxidation of methane would be expected to decrease its concentration, such reductions might be obscured due to the dynamic

biogeochemical reactions that occur within each well. It is possible that the methane fluctuations are related to water level fluctuations, both seasonal and pumping induced, and by localized water level declines triggered by recent drought conditions. The lack of correlation between methane and isobutane may be related to the efficient and complete methanotrophic oxidation of isobutane to TBA; however, additional research would be needed to verify these observations.

The evidence for methanotrophic methane oxidation from stable isotope and isobutane data is further corroborated by results from microbiological sampling and testing (Fig. 5). Microbiological samples were collected

Fig. 4 TBA concentrations co-varied with dissolved methane over approximately 7 years for Water Well A

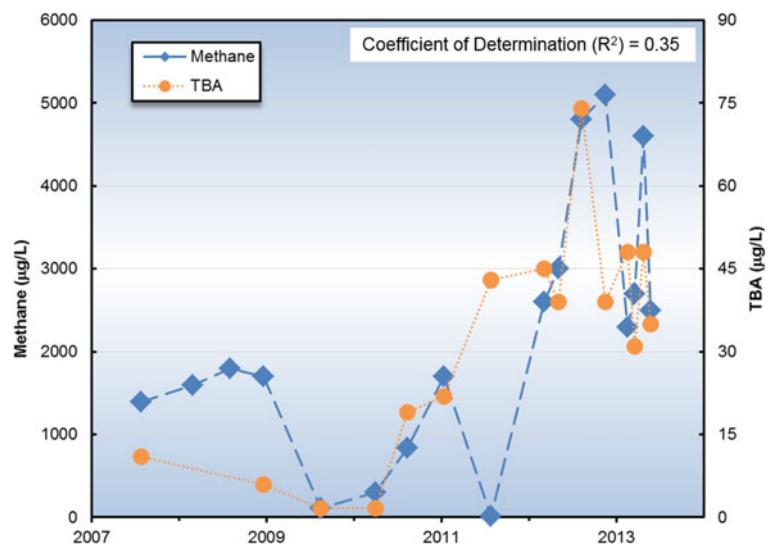
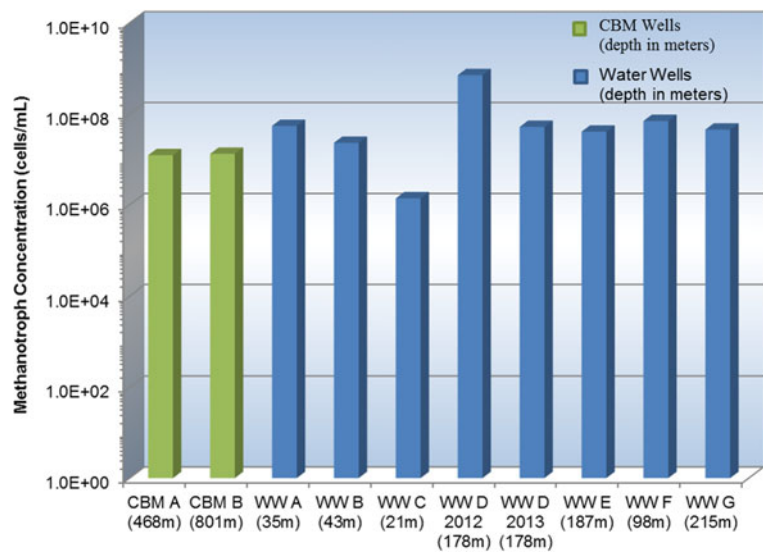


Fig. 5 Methanotroph concentrations for two CBM well (CBM A and B) and eight water well (WW) samples



from two CBM wells and five water wells, including WWA, in the very western portion of the site in Township 32S; Range 68 W (T32S R68W), and from three water wells to the far north in Township 29S; Range 67 W (T29S R67W) (Fig. 2). Enumeration of methanotrophs in the collected samples suggests the presence of a viable microbial community in each well (CBM wells $n=2$, $1.23\text{E}+07$ to $1.31\text{E}+07$ cells/ml, and water wells $n=8$, $1.38\text{E}+06$ to $7.07\text{E}+08$ cells/ml) (Fig. 5). Methanotrophs can oxidize natural gas aerobically or anaerobically. The magnitude of methanotroph cell counts in the considerably deeper and relatively oxygen-deficient CBM wells compared to shallow water wells is an indication that either (1) anaerobic oxidation is occurring in the CBM wells or (2) the low levels of oxygen (or other electron acceptors) in the CBM wells are adequate to support aerobic oxidation. Aerobic methanotroph activity at low oxygen levels has been documented in other studies (Byl and Williams 2000; Kotelnikova 2002). It may be possible that the primary form of CBM drilling in the Raton Basin, air rotary, along with water level declines associated with depressurization has resulted in the introduction of low levels of oxygen sufficient to support aerobic processes. Whether or not isobutane oxidation to TBA is occurring, and by what mechanism, is currently unknown and requires additional studies. However, evidence suggests that the oxidation of isobutane is a possible source of TBA in local groundwater. Microbial concentrations in groundwater from bedrock aquifers have been reported to range from $1\text{E}+03$ to $1\text{E}+07$

cells/ml which is comparable to methanotrophic concentrations ($1\text{E}+06$ to $1\text{E}+08$) observed in the Raton Basin CBM and water wells (Byl and Williams 2000; Newby et al. 2004). In addition to positive results for methanotrophs, DNA and mRNA results of the key enzymes involved in oxidation processes, sMMO and pMMO, were also detected in a sample collected from WWA. The DNA sMMO and pMMO results were $2.53\text{E}+03$ and $1.71\text{E}+02$ cells/ml, respectively. The mRNA sMMO was detected at a value of $2.53\text{E}+03$ cells/ml, while mRNA of pMMO was below detection ($<5.00\text{E}+00$ cells/ml). The pMMO is found in Type I, II, and X methanotrophs, while sMMO is primarily observed in methanotroph Types II and X. These results indicate active methanotroph metabolism. However, there was no clear correlation of methanotroph cell counts with well depth or concentrations of dissolved methane or TBA, with the exception of overall lower TBA and isobutane concentrations in CBM wells and the ubiquitous presence of methane. Thus, even though viable methanotrophic organism communities are present, production of TBA might be oxygen-limited in the deeper CBM wells. Additional investigation is needed to elucidate the biogeochemical interrelationships and pathways in more detail.

Evaluation of potential human health risks of TBA in Raton Basin wells

Limited information is available regarding the toxicity of TBA in humans, and no relevant

epidemiological studies were available in the peer-reviewed literature. Human volunteers have been reported to experience slight erythema and hyperemia when TBA was applied to intact skin and other human health effects included. Exposure to unquantified high TBA concentrations was reported to cause eye, nose, and throat irritation, headache, nausea, fatigue, and dizziness (Clark 2002). To-date, all of the TBA toxicology studies have been based on animal data including the comprehensive 2-year chronic toxicity and carcinogenicity studies conducted by the NTP (Cirvello et al. 1995; NTP 1995). Rats and mice in these studies were provided drinking water containing high concentrations of TBA (1,250,000 to 5,000,000 µg/l for male rats; 2,500,000 to 10,000,000 µg/l for female rats; 5,000,000 to 20,000,000 µg/l for mice of both genders). The rat kidney was the major target organ in both genders, and significantly increased incidence of combined adenomas and carcinomas was reported in the mid-dose group of male rats. Based on these results, the NTP concluded that there is “some evidence” of carcinogenicity in male rats exposed to TBA via drinking water (Cirvello et al. 1995; NTP 1995). In chronic studies with mice, the incidence of thyroid follicular cell adenomas was significantly increased in high-dose females. Based on these results, the NTP concluded that there is “some evidence” of carcinogenicity in female mice (Cirvello et al. 1995; NTP 1995). Robust summaries including all required toxicological endpoints were submitted by industry to EPA under the HPV Challenge Program in 2004 (Propylene Carbonate/t-Butyl Alcohol HPV Committee 2004), and EPA’s Screening-Level Hazard Characterization (EPA 2009) determined that there were no toxicological data gaps. However, neither EPA nor IARC has developed consensus toxicity criteria for TBA.

In the absence of consensus toxicity criteria, a number of state regulatory agencies have developed groundwater screening levels for TBA. These values have various underlying toxicological bases and span several orders of magnitude, even when based on the same toxicological endpoint (Fig. 6). Most importantly, none were derived using current EPA methodology with respect to toxicological endpoint selection, methods for calculating human toxicity criteria and developing risk-based screening levels in drinking water (EPA 2005, 2007, 2012a, 2015b).

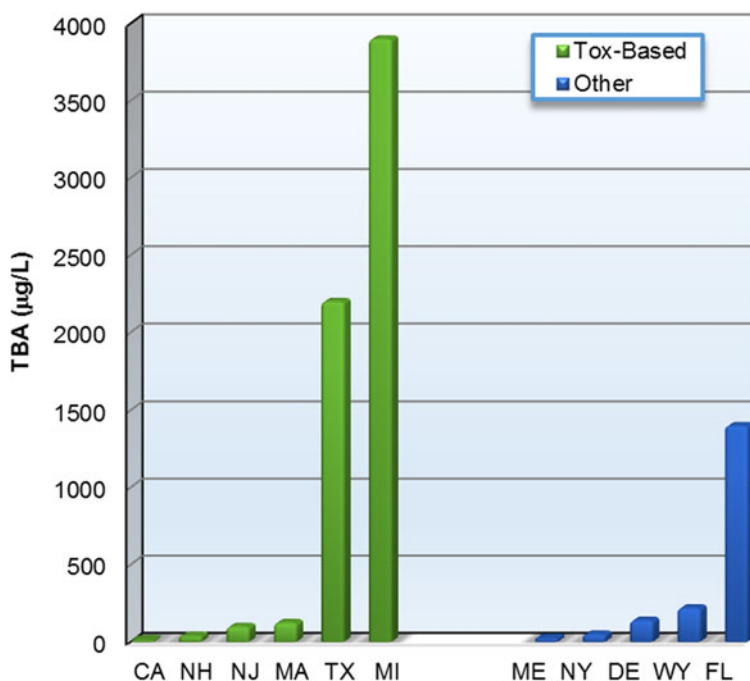
The most stringent state groundwater guideline, 12 µg/l, was calculated by California’s Office of Environmental Health Hazard Assessment (1999) based on male rat kidney tumors in the NTP 2-year drinking water study (Cirvello et al. 1995; NTP 1995). Because the TBA-induced male rat kidney tumor has since been shown to be irrelevant to humans (Alison et al. 1994; McGregor 2010; Hard et al. 2011, 2013), the California interim groundwater guideline of 12 µg/l is inappropriate for use in TBA risk assessment, with respect to human health.

NSF International (NSF International 2003) and the American Petroleum Institute (API) (API 2005) conducted comprehensive reviews of TBA toxicity data and developed human toxicity criteria in accordance with contemporary EPA guidance. Neither group considered male rat kidney tumors or female mouse thyroid follicular cell adenomas to be relevant endpoints for human health risk assessment. Both groups determined that thyroid follicular cell hyperplasia, which appears to be a key precursor event for adenoma development in female mice, is potentially relevant to humans and provides a conservative (more likely to over- than underestimate human risk) basis for development of human toxicity criteria. API developed the lower of the two risk-based screening levels for drinking water, 8000 µg/l (API 2005). Although not fully consonant with current EPA guidance (EPA 2005, 2007, 2012a, 2015b) and predating relevant toxicological information concerning carcinogenic mode of action (e.g., Meek et al. 2014), this screening level is considered appropriate for purposes of preliminary evaluation of potential human health risks associated with TBA in Raton Basin wells. As the highest water well TBA concentration in this study (395 µg/l) is an order of magnitude below this risk-based screening level, no adverse effects on human health are expected from TBA if the groundwater is used as a potable water supply.

Conclusions and recommendations

This forensic analysis afforded an opportunity to investigate potential linkages between TBA occurrence and CBM operations. The available evidence from the Raton Basin suggests that TBA is a non-point source contaminant, with detections likely due to a

Fig. 6 State drinking water criteria for TBA. *Green bars* indicate values based on TBA toxicological criteria; *blue bars* indicate non-specific values. Data from the New England Interstate Water Pollution Control Commission (2006)



combination of factors including widespread commercial use and biogenic production. Analysis of historic and supplemental monitoring data indicates that the temporally and spatially irregular detections of TBA were not associated with the direct use of, or contamination by reformulated gasoline fuel oxygenates, drilling or HF fluids, CBM operations, or any other specific plumes or potential sources. Testing of common water well products indicates the potential for low levels of TBA in water wells (but not CBM wells) from their use. The presence of significant and active methanotrophic activity along with coexistence of isobutane with methane, and isotopic evidence of oxidation trends, provides preliminary evidence that biogenic production of TBA may be occurring in some wells. From a public health perspective, the levels of TBA that were detected in water wells appear to be below the levels of toxicological concern. Because the majority of available data were historical and not targeted at identifying a source of TBA, data gaps exist and additional testing is needed to confirm the source of TBA in the different wells, including the potential for biogenic production of TBA. Additional testing to evaluate potential biogenic TBA production includes microbial analyses of groundwater samples from more wells and targeted analysis of MMO to identify active metabolic processes. Supplemental analyses of the co-occurrence of TBA and other

organics, dissolved gases, microbiological parameters and isotopes of methane could provide additional insight into the natural variability of these parameters and the conditions under which microbial production may occur. Additionally, field monitoring of dissolved oxygen and/or oxidation reduction potential could provide useful data for identifying or confirming the likelihood of different potential microbial pathways. Laboratory-scale studies with soil and water collected from Raton Basin well sites could help to elucidate mechanisms and refine field investigations. Isotope analysis of isobutane may also be possible, and it is hypothesized that oxidation of isobutane would result in a fractionation of the carbon isotopes. If the fractionation pattern is significant and can be identified in a laboratory study, then laboratory results could be used to assess isobutane carbon isotope values identified in field samples. Finally, extensive testing of common well completion materials used in water wells should be carried out and documented. Only two water well completion materials were collected and analyzed during this investigation, and additional testing may well reveal more products that could be sources of low levels of TBA.

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Compliance with ethical standards

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