

Nature and Estimated Human Toxicity of Polar Metabolite Mixtures in Groundwater Quantified as TPHd/DRO at Biodegrading Fuel Release Sites

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Abstract

Polar metabolites resulting from petroleum biodegradation are measured in groundwater samples as TPHd unless a silica gel cleanup (SGC) is used on the sample extract to isolate hydrocarbons. Even though the metabolites can be the vast majority of the dissolved organics present in groundwater, SGC has been inconsistently applied because of regulatory concern about the nature and toxicity of the metabolites. A two-step approach was used to identify polar compounds that were measured as TPHd in groundwater extracts at five sites with biodegrading fuel sources. First, gas chromatography with mass spectrometry (GC-MS) was used to identify and quantify 57 individual target polar metabolites. Only one of these compounds—dodecanoic acid, which has low potential human toxicity—was detected. Second, nontargeted analysis was used to identify as many polar metabolites as possible using both GC-MS and GC×GC-MS. The nontargeted analysis revealed that the mixture of polar metabolites identified in groundwater source areas at these five sites is composed of approximately equal average percentages of organic acids, alcohols and ketones, with few phenols and aldehydes. The mixture identified in downgradient areas at these five sites is dominated by acids, with fewer alcohols, far fewer ketones, and very few aldehydes and phenols. A ranking system consistent with systems used by USEPA and the United Nations was developed for evaluating the potential chronic oral toxicity to humans of the different classes of identified polar metabolites. The vast majority of the identified polar metabolites have a "Low" toxicity profile, and the mixture of identified polar metabolites present in groundwater extracts at these five sites is unlikely to present a significant risk to human health.

Introduction

Total petroleum hydrocarbons (TPH) has long been a routinely required groundwater monitoring parameter at petroleum release sites. For the semi-volatile (or extractable) fraction, a common analytical approach is to use USEPA Method 3510C for solvent extraction, followed by USEPA Method 8015B/C or equivalent (gas chromatography with flame ionization detection [GC-FID]) for quantitation. The purpose of the analysis, called either TPH as diesel (TPHd) or diesel-range organics (DRO), is to measure C10 to C28 hydrocarbons dissolved in the groundwater. The TPHd concentration is then usually compared to hydrocarbon-based regulatory criteria. However, neither Method 3510C nor 8015B/C is specific for hydrocarbons, and the TPHd analysis actually measures all extractable organics within the prescribed boiling-point range (170 to 430 °C in the case of Method 8015B/C).

In order to compare sample TPHd results to hydrocarbon-based water quality objectives, a silica gel cleanup (SGC) must be applied to the sample extract prior to analysis. This was documented in a study of 21 sites (Zemo and Foote 2003), where the application of SGC to the sample extract prior to analysis for TPHd showed that the majority of samples with elevated concentrations of TPHd in groundwater at sites with biodegrading petroleum sources were composed almost entirely of dissolved polar, nonhydrocarbon compounds and not dissolved diesel-range hydrocarbons. The polar compounds were typically found in groundwater directly within the source area and downgradient from biodegraded petroleum; therefore, it was concluded that they were most likely biodegradation metabolites. This finding was confirmed by Lundegard and Sweeney (2004), Haddad et al. (2007), and Lang et al. (2009). These studies showed that the concentration of polar metabolites, quantified as TPHd, typically ranged from 100s to 10,000s micrograms per liter (µg/L), with a maximum of about 100,000 µg/L.

In addition to presumed biodegradation metabolites, other studies have demonstrated that nonhydrocarbons measured as TPHd may include natural organics, laboratory or sample equipment artifacts (e.g., phthalates), or nonpetroleum chemicals (Zemo et al. 1995; Uhler et al. 1998). Zemo and Foote (2003) recommended the routine use of SGC for TPHd analysis to facilitate comparison of the concentration of the hydrocarbons in the sample to hydrocarbon-based regulatory

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criteria. However, over the last decade SGC has been applied inconsistently because of regulatory concern about the nature and toxicity of the polar metabolites. This regulatory approach can result in expensive and potentially unnecessary additional investigation or remediation, or protracted site closures.

Polar compounds naturally present in crude oil (nitrogen-, sulfur-, or oxygen-containing heteromolecules) are largely eliminated in the refining processes used to produce gasoline, jet fuel, and diesel because they are deleterious to fuel performance (Hamilton and Falkiner 2003; Strauss 2003; Westbrook and LeCren 2003). Therefore, the water-soluble fraction of these fresh unbiodegraded fuels typically contains few or virtually no oxygen-containing polar compounds, except for chemicals such as oxygenates purposely added to the fuel. The presence of a high proportion of oxygen-containing polar compounds other than additives at fuel release sites is direct evidence that biodegradation is occurring (Barcelona et al. 1995; Beller et al. 1995; Beller 2002). The oxidative biodegradation of petroleum hydrocarbons has been studied extensively, and intrinsic and enhanced biodegradation are widely accepted remediation methods for petroleum releases (Wiedemeier et al. 1995; USEPA 1999). Both aerobic and anaerobic biodegradation processes involve transformation of the hydrocarbon molecules by sequential oxidative reactions, ultimately producing small organic acids, which are transformed to carbon dioxide and water (Atlas 1981; Dragun 1988; Cozzarelli et al. 1994; Barcelona et al. 1995; Beller et al. 1995). Intermediate steps result in the formation and subsequent biodegradation of oxygen-containing polar compounds (metabolites) that can be categorized by chemical structure into five families: acids/esters, alcohols, phenols (from aromatic hydrocarbons only), aldehydes, and ketones (Healy et al. 1980; Harayama et al. 1999; Griebler et al. 2004; Young and Phelps 2005; Chakraborty and Coates 2005; Callaghan et al. 2006; Geig et al. 2009). These five families can be further subdivided into structural classes (normal and branched, cyclic, aromatic, bicyclic and polycyclic aromatic) based on precursor hydrocarbon structures, which results in a total of 22 structural classes for the potential polar metabolites as shown in Table 1. Individual metabolites are transient. Because fuels are mixtures of hundreds of individual hydrocarbons, thousands of individual transient polar metabolites are possible.

Identifying polar metabolites produced by biodegrading fuels in environmental samples using traditional analytical techniques is challenging because of the large number of potential compounds that can be present at low concentrations, that is, $\mu\text{g/L}$. Most work on metabolites has focused on identifying organic acids in groundwater using derivatization procedures followed by GC (Barcelona et al. 1995; Cozzarelli et al. 1995; Beller 2002; Martus and Puttmann 2003; Alumbaugh et al. 2004; McKelvie et al. 2005). Using GC-MS, Langbehn and Steinhart (1995) identified acids and ketones in soil affected by biodegrading diesel. Recently, the availability of two-dimensional separation techniques, such as two-dimensional gas chromatography with time-of-flight mass spectrometry (GC \times GC-MS), has increased the ability to characterize an increased number of compounds. GC \times GC performs complimentary separations in two dimensions simultaneously and results in a greater

Table 1
Structural Classes of Polar Metabolites from Biodegradation of Fuels and Expected Chronic Oral Toxicity to Humans

Polar Family	Specific Structural Class	Expected Chronic Oral Toxicity to Humans
Alcohols (and diols)	Alkyl alcohols	Low
	Cycloalkyl alcohols	Low
	Bicyclic alkyl alcohols	Low
	Aromatic alcohols	Low
	Polycyclic aromatic alcohols	Low to Moderate
Acids (and esters)	Alkyl acids	Low
	Cycloalkyl acids	Low
	Bicyclic alkyl acids	Low
	Aromatic acids	Low
Ketones	Polycyclic aromatic acids	Low to Moderate
	Alkyl ketones	Low to Moderate
	Cycloalkyl ketones	Low
	Bicyclic alkyl ketones	Low
	Aromatic ketones	Low to Moderate
Aldehydes	Polycyclic aromatic ketones	Low to Moderate
	Alkyl aldehydes	Low to Moderate
	Cycloalkyl aldehydes	Low to Moderate
	Bicyclic alkyl aldehydes	Low to Moderate
	Aromatic aldehydes	Low to Moderate
Phenols	Polycyclic aromatic aldehydes	Low to Moderate
	Alkyl phenols	Moderate
	Phenol	Low

Notes: Toxicity ranking system and criteria for Expected Chronic Oral Toxicity to Humans are explained in the body of the article. Low: $\text{RfD} \geq 0.1$; Low to Moderate: $0.1 > \text{RfD} \geq 0.01$; Moderate: $0.01 > \text{RfD} \geq 0.001$.

sensitivity than traditional one-dimensional GC (Marriott et al. 2012; Ryan and Marriott 2003). Mao et al. (2009) identified acids/esters, alcohols, phenols, aldehydes, and ketones in a laboratory-generated leachate of diesel-containing soil undergoing aerobic biodegradation using high-performance liquid chromatography followed by two-dimensional gas chromatography with flame ionization detection (HPLC-GC \times GC-FID) and GC \times GC-MS.

The purpose of this study is to characterize the chemical structure and potential toxicity of polar metabolites measured as TPHd in groundwater samples at five fuel-impacted sites. A two-step analytical approach was used, in which groundwater samples were analyzed quantitatively for a target list of potential individual polar metabolites for which toxicity information and analytical standards were available using traditional GC-MS analyses, and additional polar compounds were tentatively identified using nontargeted (Hoh et al. 2012) GC-MS and GC \times GC-MS techniques. The potential human toxicity of the mixtures of identified polar metabolites was then assessed. Due to

the transient nature of the polar metabolites and uncertainty associated with specific isomeric identification, the nontargeted investigation focused on the five families and 22 structural classes presented in Table 1, rather than on individual compounds.

Methods

Selection of Target Compounds

An initial list of individual “target” polar compounds was developed for quantitative analysis based on a review of the literature, first by identifying the chemical structures of expected polar metabolites, and then by collecting existing toxicology information for individual compounds with the identified structures. USEPA and other regulatory program databases served as the primary sources for toxicology information. All potential polar metabolites for which reference doses (RfDs) are listed on the USEPA table of regional screening levels (USEPA 2012) were included. Additional compounds for which sufficient toxicology information was available in the scientific literature were added to the list, to make certain that representative compounds from each structural class were included wherever possible. An initial list of 83 compounds was reduced to 65 based on the availability of chemical standards, and to a final list of 57 compounds based on compound boiling points and solvent extraction results (described later) (Table 2). The final list of compounds consisted of 11 organic acids/esters, 14 alcohols, 12 phenols, 11 ketones, and 9 aldehydes.

Collection of Groundwater Samples

Groundwater was sampled at five fuel terminals (four active and one inactive) with known historical fuel releases of both gasoline and diesel. All five sites are located in California; four sites are in upland settings, and one site (Site 5) is immediately adjacent to marine surface water. Multiple years of TPHd data were available from groundwater monitoring at each site, and plume configurations were known. Monitoring wells were selected for sampling at each terminal to represent source and downgradient areas. For the purpose of this study, source-area wells were those where free product or sheen had been observed within the past 10 years. However, because these are large terminal sites, it is likely that multiple source areas exist along the groundwater flow paths between wells. To account for this, the presence of relatively elevated methane in a groundwater sample was used as a secondary criterion for defining source areas, reflecting the fact that source areas are often methanogenic (Wiedemeier et al. 1995). Downgradient wells are those generally hydraulically downgradient of the source area with relatively lower or no methane, but still within the TPHd plume. All monitoring wells were screened at or near the water table; the depth to the water table was generally shallow (<40 feet) and seasonally fluctuating.

A total of 22 groundwater samples were collected for the study. Samples were collected at each site during a quarterly monitoring event by the same sampling contractor and by routinely used methods, in order to replicate as closely as possible the previous TPHd results. Most wells were purged

Table 2
List of Target Polar Compounds for GC-MS
Quantitative Analysis

Analyte	
Alcohols	Ketones
Cyclopentanol	5,6-Dimethoxy-1-indanone
1-Decanol ¹	2-Dodecanone ¹
2,4-Dimethyl-3-pentanol	9-Fluorenone ¹
1-Dodecanol	2-Hexanone
1-Heptanol ¹	2-Methyl-1-indanone
1-Hexanol ¹	3-Methylacetophenone
2-Methyl-1-pentanol	4-Methylacetophenone
2-Methyl-2-hexanol	2-Nonanone ¹
1-Nonanol ¹	2-Pentadecanone
1-Octanol ¹	2,6,8-Trimethyl-4-nonanone
2-Phenylethanol (benzene ethanol)	2-Undecanone ¹
1-Tetradecanol [50]	<i>Phenols</i>
2,4,4-Trimethyl-1-pentanol ¹ [50]	4-Tert-amylphenol ¹
1-Undecanol ¹	4-Tert-butylphenol ¹
<i>Acids</i>	4-Cumylphenol ¹
Cyclohexaneacetic acid ¹	2,4-Dimethylphenol ¹
Cyclohexanecarboxylic acid [100]	2,6-Dimethylphenol
Dodecanoic acid	3,4-Dimethylphenol
2-Hydroxy-1-naphthoic acid	2-Methylphenol
1-Hydroxy-2-naphthoic acid [50]	4-Methylphenol
4-MCacetic acid	4-Octylphenol
4-Methyl hexanoic acid ¹	4-Pentylphenol ¹
Pentanoic acid [250]	2-Phenylphenol
Phenylacetic acid [50]	2,3,5-Trimethylphenol ¹
Undecanoic acid [100]	
Methylbenzoate (ester) ¹	<i>Compounds not included due to BP << 170 °C</i>
<i>Aldehydes</i>	1-Propanol
Benzaldehyde	Formaldehyde
Decanal ¹	Propanal
Dodecanal ¹	<i>Compounds not included due to poor extraction</i>
Heptanal ¹	Catechol
Hexanal	Hydroquinone
Nonanal ¹	1,5-Pentanediol
Octanal ¹	2-Methyl resorcinol
3,5,5-Trimethylhexanal ¹	2,5-Dimethyl resorcinol
Undecanal ¹	

Notes: Limit of Quantitation is 10 µg/L unless bracketed value is shown.

¹Standard also run on GCxGC-MS (27 on this table plus nonylphenol; see text).

and sampled using disposable bailers and a few wells were purged using a centrifugal pump and sampled with a disposable bailer. Groundwater from each well was collected into

four, unpreserved 1 L amber bottles. Samples to be analyzed for natural attenuation parameters (nitrate, ferrous iron, sulfate, and methane) were collected into appropriate containers and preserved as required. One blind field duplicate sample was collected at each site. Samples from each site were shipped separately to a commercial laboratory under chain-of-custody procedures.

Chemical Analysis

Sample analyses included both targeted quantitative and nontargeted qualitative methods. To replicate as closely as possible the mixture of organic compounds present in the previous sample extracts analyzed for TPHd at the study sites, and to be consistent with previous analytical procedures used for the study sites, the state-certified commercial laboratory that routinely performs the regulatory compliance analyses for these sites was used to (1) extract all samples, (2) perform the SGC, and (3) perform the quantitative analysis of the samples.

The laboratory combined all four 1 L amber bottles for each well prior to extraction to homogenize the sample, and then split the sample into four 1 L portions. The samples were extracted using methylene chloride (DCM) in accordance with USEPA Method 3510C. Extracts were analyzed for: (1) TPHd using USEPA Method 8015B without and with a column SGC (based on USEPA Method 3630C), (2) the 57 target polars using a modification of USEPA Method 8270C, and (3) an open-scan GC-MS library search with reporting of the top 40 tentatively identified compounds (TICs). In addition, the silica gel column was eluted with methanol in an attempt to remove the polars, and the methanol eluate was analyzed for the target polars and the GC-MS library search. Finally, aliquots of all extracts and the methanol eluate for each well were sent to the Chevron Energy Technology Company (CETC) in-house laboratory for analysis by two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-MS) to qualitatively identify polar compounds.

Targeted Quantitative Analyses

TPHd by USEPA Method 8015B was performed on the DCM extract and DCM with SGC (discussed below) extract for each sample by the state-certified commercial laboratory using GC-FID in accordance with USEPA SW-846 methods. The carbon range for quantitation was C10 to C28 (boiling points of 170 to 430 °C), and diesel fuel #2 was used as the standard. The internal method recovery surrogate was *ortho*-terphenyl (OTP). Gas chromatograms were provided for each sample.

Silica gel cleanup was performed on one of the duplicate extracts for each sample according to USEPA Method 3630C. Briefly, a glass column was packed with 10 g of activated silica gel, the packed column was pre-rinsed with pentane, a capric acid reverse surrogate was spiked into the DCM extract, and the DCM extract was placed onto the silica gel column. The column was then eluted with a DCM:pentane mixture. Acceptable retention of polars onto the silica gel was evaluated for each sample, as defined by a capric acid recovery range of 0% to 1%. Recovery greater than 1% indicates that the removal of polars may not have

been complete. Acceptable recovery of the hydrocarbons in the cleaned-up extract was evaluated for each sample batch by laboratory control samples (LCSs) and LCS duplicates (LCSDs) spiked with diesel fuel.

Extracts and the methanol eluate for each well were analyzed quantitatively at the commercial laboratory by GC-MS using modified USEPA Method 8270C for the target polars. The GC-MS was equipped with a 20 m × 0.18 mm × 0.18 μm df DB-5MS column and a split/splitless injector. A 1 mL splitless injection was made into the GC. Standards for 65 target polar compounds (Ultra Scientific, North Kingstown, RI) were prepared in DCM and methanol. A 5-point calibration was constructed to determine the method detection limit, relative response factor, and amenability of each compound to analysis on a GC. The chromatography study and an extraction efficiency study showed that 8 of the 65 target polars would not be included in a TPHd quantitation due to either boiling points below 170 °C or inefficient extraction using Method 3510, and these were dropped from further evaluation. The final list of 57 target polars, their respective limits of quantitation (LOQs), and the eight compounds dropped, are shown in Table 2. Except for five acids and two alcohols, the LOQs for all of the analytes are 10 μg/L. QA/QC was performed using LCS spikes/LCS duplicates and recovery surrogates.

Nontargeted Qualitative Analyses

An automated mass spectral library search function was used by the commercial laboratory as part of its GC-MS analysis to tentatively identify the compounds present. Any peak identified with a mass spectral match greater than 75% was assigned the compound name. The laboratory reported the top 40 TICs for each sample.

All DCM extracts and the methanol eluate for each well were analyzed at the CETC in-house laboratory using comprehensive GC×GC-MS to tentatively identify the compounds present. Two microliters of the extract were injected into the GC×GC. The first column was a 40 m × 0.18 mm × 0.2 mm RTX-1, and the second column a 1.5 m × 0.1 mm × 0.1 mm BPX-50. An automated search was performed of the National Institute of Standards and Technology mass spectral library, and peaks with a quantitative signal-to-noise ratio of greater than or equal to 5 and spectra that matched with a mass spectral similarity value of at least 750 were tentatively assigned compound names. The match similarity value of 750 was set as a balance between the confidence in identification and the signal-to-noise ratio of compounds present in the samples. Any compound with a match less than 750 was considered to be an unknown. For this study, only the total ion count chromatogram was used for the library search, and individual mass channels were not studied.

The GC×GC-MS results are not quantitative; however, a subset of 28 standards (Table 2) were injected into the GC×GC to evaluate (1) the estimated LOQ for the TICs, and (2) compare the relative response factors for the various classes of compounds, to ascertain the differences in detector responses. Based on the standards run, the LOQ for the TICs ranged from 1 to 11 μg/L, with all but two TICs ranging from 1 to 5 μg/L. The average relative response factor for the tested standards in each of the five polar families in

the C8 to C12 carbon range varied by only a factor of about 2, signifying that a direct comparison of response could be made for the majority of the classes.

To be comprehensive, for each well, all TICs above the signal-to-noise ratio threshold were reported for each extract and the methanol eluate. The results for the extracts and eluate were compared, and the unique polars were identified for each well. This compilation resulted in a larger population of TICs than just the methanol eluate from the silica gel column, captured as many TICs as possible from each well, and did not require reliance on the unknown effectiveness of the commercial lab's methanol elution to represent the polar fraction. The compiled unique TICs in each well were assigned to their respective polar families and structural classes, and the results were tallied.

Human Toxicity Evaluation

A conservative relative ranking system for the potential chronic human toxicity of polar compounds was developed based on agency-derived toxicity criteria and, in their absence, toxicity information available in the open scientific literature. Human health risk assessments for contaminants at remediation sites are generally screened using toxicity criteria known as RfDs, defined as “an estimate of a daily oral exposure for a given duration to the human population

(including sensitive subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime” (USEPA 1989). The USEPA's regional screening level (RSL) and the Texas Commission on Environmental Quality Risk Reduction Program's protective concentration level (TCEQ PCL) tables were thus reviewed to identify potential polar metabolites for which agency-developed RfDs were available (USEPA 2012a; TCEQ 2012). Equivalent risk-based tap water concentrations for the range of identified RfDs were derived using the USEPA equation used to derive tap water RSLs (SL_{water-nc-ing}) (USEPA 2012b). The RfDs and calculated tap water equivalent concentrations for potential individual polar metabolites identified from these literature sources are presented in Figure 1. Note that the individual compounds shown in Figure 1 were not necessarily identified in groundwater samples during this study. Note also that the calculated tap water equivalent concentrations shown in Figure 1 are provided simply for context, for the benefit of those less familiar with RfDs. This study did not develop “screening levels” for the polar metabolites in groundwater for site management purposes (which would be a significantly more complex effort than appropriate for this study), and does not recommend the use of the concentrations shown in Figure 1 as “screening levels” for mixtures of polar metabolites in groundwater.

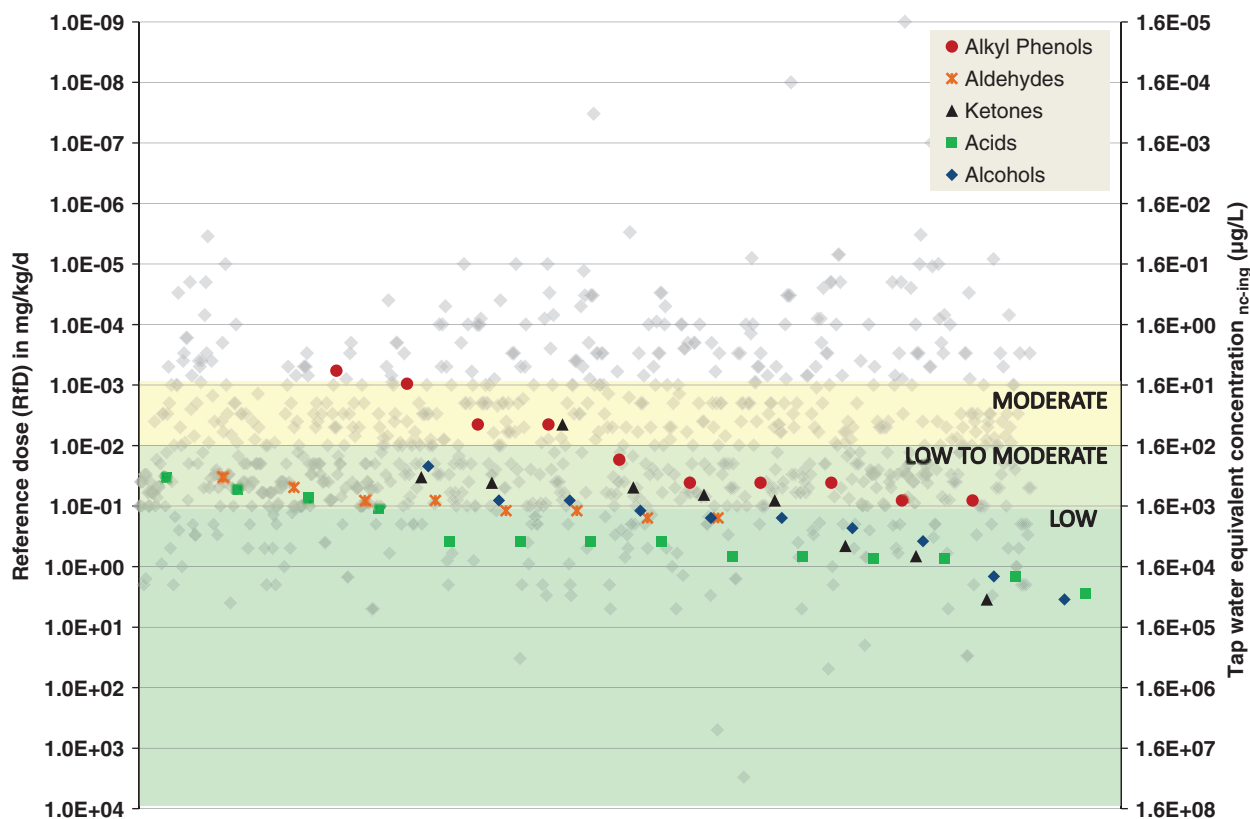


Figure 1. RfDs for polar metabolite families, toxicity rankings, and calculated tap water equivalent concentrations. Background (gray) dots represent the entire universe of chemicals for which RfDs have been established, as listed on the USEPA RSLs Table or the Texas CEQ PCLs Table (including pesticides, chlorinated compounds, etc.). Colored symbols are individual chemicals with RfDs that are potential polar metabolites within each of the five polar families. The individual chemicals shown here were not necessarily identified in this study, but are representative of the polar family. The tap water equivalent concentrations were calculated from the RfDs and are shown for context only. These values should not be construed as regulatory “screening levels” for mixtures of polar metabolites in groundwater.

A conservative RfD-based toxicity ranking system was then developed consistent with similar systems developed by USEPA and the United Nations for use in other non-RfD based regulatory programs (Tiwary et al. 2013). Summary rankings of “Low,” “Low to Moderate,” and “Moderate” were assigned to the identified RfDs. Figure 1 shows that polars with RfDs ≥ 0.1 mg/kg/d were defined as being of Low toxicity. Polars in the range of $0.1 > \text{RfD} \geq 0.01$ mg/kg/d were defined as Low to Moderate toxicity, and those in the range of $0.01 > \text{RfD} \geq 0.001$ mg/kg/d were defined as Moderate toxicity. These three ranking groups include essentially all the potential polar metabolites for which RfDs were available.

These summary rankings are consistent with USEPA (USEPA OPPT 2009, 2012) criteria and the United Nations’ Globally Harmonized System of Classification and Labeling of Chemicals (UNECE 2011). For example, under the USEPA Office of Pollution Prevention and Toxics (OPPT) Chemical Assessment and Management Program, a chemical that produces a lowest observed adverse effect level (LOAEL) of >100 mg/kg/d in 13-week or 90-d repeated-dose toxicology studies is defined as presenting low chronic toxicity potential. For purposes of this study, the LOAEL-based criterion of 100 mg/kg/d can be converted to an RfD-equivalent criterion of 0.1 mg/kg/d by applying an uncertainty factor of 1000, consistent with USEPA procedures (USEPA 1989). The toxicity ranking system developed in this study is consistent with and generally more conservative than the criteria put forth by the USEPA OPPT approach as shown in Table 3. For example, specific alkyl phenols such as xylenols and cresols that are classified as “Low to Moderate” or “Moderate to High” under our ranking system would be classified as presenting “Low” chronic toxicity under the USEPA OPPT system.

The Low, Low to Moderate, and Moderate summary toxicity rankings were assigned to all 22 structural classes of potential polar metabolites as shown in Table 1. This step

required additional toxicity information in some cases. For compounds that did not have an agency-derived RfD, but for which published toxicology test data were available either for the chemical itself or for a structurally and/or functionally related chemical, a provisional RfD was derived based on the reported no-adverse-effect level from repeated-dose animal studies and applying an uncertainty factor of 1000. An overall ranking was assigned to each structural class based on the collective weight of the evidence for representative compounds in that class.

Finally, as a validation exercise, USEPA summary documents were reviewed for selected compounds with known RfDs, and the corresponding toxicity ranking assigned in this study was compared to USEPA opinions expressed in their documents. For example, propanoic acid (RfD >0.1 mg/kg/d ranked as “Low” toxicity) belongs to the *n*-(alkyl) carboxylic acid category summarized by USEPA to have “low repeated-dose, reproductive and developmental toxicity” (USEPA 2008a). Similarly, alkyl phenols with RfDs ranging from >0.1 mg/kg/d (“Low” toxicity) to 0.001 mg/kg/d (“Moderate” toxicity) are summarized as having “low to moderate hazard potential for acute and repeated-dose toxicity” (USEPA 2008b).

Results and Discussion

Results for the polar compounds that are potential metabolites, which were the vast majority of the polar TICs in these samples, are presented and discussed herein. Other polar compounds that were infrequently identified and that are not potential metabolites (chlorinated compounds, benzothiofenenes, plasticizers, pesticides) are not presented or discussed. These nonmetabolites were not a significant component of these samples either in terms of numbers of TICs or detector response (peak area), with the exception of the plasticizers in a few samples. Except for the

Table 3
Comparison of Toxicity Rankings Between USEPA/UN Systems and This Study

Polar Compound	LOAEL	EPA/UN Ranking Based on LOAEL	RfD	RfD-Based Ranking (This Study)
Dimethylphenol, 2,4-	180	Low	0.02	Low to Moderate
Dimethylphenol, 2,6-	400	Low	0.0006	Moderate to High
Cresol, <i>m</i> -	150	Low	0.05	Low to Moderate
Cresol, <i>o</i> -	175	Low	0.05	Low to Moderate
Phenol	280	Low	0.3	Low
Benzaldehyde	400	Low	0.1	Low
Acetone	1700	Low	0.9	Low
Methyl ethyl ketone (2-Butanone)	1771	Low	0.6	Low
Acrylic acid	240	Low	0.5	Low
Ethyl acetate	3600	Low	0.9	Low
Methanol	2500	Low	0.5	Low
Butanol, <i>N</i> -	500	Low	0.1	Low
Isobutyl alcohol	1000	Low	0.3	Low

benzothiophenes, which were very infrequently identified, the nonmetabolites have no relationship to the presence of the residual fuel at these sites and thus would have no role in risk management associated with the residual fuel.

Detailed analytical results for the commercial lab are shown in Table 4. The GC×GC-MS analytical results and toxicity evaluation results are summarized in Tables 5 and 6. Esters, which are reversibly formed from an acid and an alcohol, were classified with the acids for this study.

Based on the results for the natural attenuation parameters (Table 4), all wells were within the zone of biodegradation. The redox conditions were generally anaerobic and varied from nitrate-reducing to methanogenic, depending on the site and the relative position of each well with respect to the source area.

TPHd Results

All TPHd results (Table 4), including those with SGC, were within the laboratory's acceptable control ranges. The TPHd concentrations for each groundwater sample were similar to previous monitoring events. The TPHd concentrations without SGC (representing all organics extracted by Method 3510C and with boiling points between 170 and 430 °C) for samples containing only dissolved organics ranged from 1000 to 8100 µg/L in source-area samples, and from 98 to 1700 µg/L in downgradient samples. A review of the chromatograms revealed that 4 of the 13 source-area samples contained a nondissolved product component (Site 2 MW-6, Site 1 MW-5A, Site 1 MW-100/5A duplicate, Site 1 MW-26A). Nondissolved product is characterized by a chromatogram with a distinctive fuel pattern (dominated by an unresolved complex mixture [UCM] in the appropriate carbon range in the case of middle distillates) and not by the individual hydrocarbon peaks that correspond to the water-soluble fraction of fuels (primarily the C14 and smaller aromatics and very small aliphatics). The inclusion of a nondissolved component was an artifact of sheen or petroleum-impacted soil particles (turbidity) in the samples caused by the act of sampling. These four samples had TPHd concentrations ranging from 2000 to 27,000 µg/L.

Except for the samples with entrained nondissolved product, the TPHd chromatograms for the study samples were all dominated by a prominent UCM that was not representative of a fuel pattern or dissolved hydrocarbon pattern but is typical for complex mixtures of polar compounds at sites with biodegrading petroleum sources (see Figure 4 of Zemo and Foote 2003). Except for Site 5 (discussed below) and samples with entrained nondissolved product, TPHd concentrations for all samples but one were reduced to nondetect (<100 µg/L) after the SGC. This indicates that virtually all of the organics in groundwater and the components of the UCM at these sites are polars, and not dissolved hydrocarbons. Based on the difference between the TPHd concentration without SGC and with SGC for each sample, the percentage of the dissolved organics that were polars ranged from 84 to 100% in source-area samples and was 100% in downgradient samples. Samples with entrained nondissolved product (Site 2 MW-6, Site 1 MW-5A, Site 1 MW-26A) had less reduction in TPHd concentration after SGC because of the hydrocarbons present.

At Site 5, the TPHd concentrations were significantly reduced after SGC, but remained above 100 µg/L. The SGC was incomplete for all of the Site 5 samples, as indicated by either a capric acid recovery greater than 1% or the chromatogram pattern. The reason for the incomplete SGC at Site 5 is unclear. Even with an incomplete SGC, the percentage of dissolved polar compounds in the Site 5 samples ranged from at least 65% to at least 91%.

Targeted GC-MS Analysis

The quantitative results from Modified EPA Method 8270C for the 57 target polars (Table 4) show that, except for 11 µg/L dodecanoic acid in one sample, none of the individual compounds were detected in any of the extracts/eluates for any groundwater sample (most reporting limits were 10 µg/L). The LCS/LCSD recoveries and the Method 8270C surrogate recoveries were generally within the laboratory's acceptable range.

Dodecanoic acid (also known as lauric acid CASRN 143-07-7) is a C12 saturated fatty acid. Based on the toxicity ranking system, this compound and its structural class (alkyl acid) are of Low toxicity. It is equally important to note that included among the 57 target polars were 12 alkyl phenols, representing the relatively more toxic polar metabolites (as shown on Figure 1), none of which were detected in any sample at a reporting limit of 10 µg/L.

Nontargeted GC-MS Analysis

Combining the GC-MS Library Search results for the DCM extracts and methanol eluates for all wells at each site, the number of polar metabolite TICs for each site ranged from 4 (Site 2) to 27 (Site 5) (Table 4). The number of unique polar metabolite TICs in a single well ranged from 1 to 8. The three Site 5 source area samples had the largest number of TICs and included organic acids, ketones, phenols, and one aldehyde. Organic acids were the only compounds tentatively identified in other samples, except for one phenol in Site 2 MW-6. Most of the organic acids were identified as "unknown carboxylic acid"; "naphthalene carboxylic acid" was identified in four samples.

Nontargeted GC×GC-MS Analysis

Because of the uncertainty associated with MS library matching, inability to distinguish among potential isomers, and lack of standard-based confirmation, specific individual TICs are not discussed in detail here but rather are reported by family and structural class. TIC concentrations could not be calculated because standards were not available, which prevented the generation of calibration curves; however, based on the 28 standards that were run, it was determined that the LOQ for a majority of the identified compounds is in the range of 1 to 5 µg/L. The GC×GC also detected several of the 57 target polar metabolites that were not detected by the traditional GC at detection limits of 10 µg/L, further suggesting that the target polars identified by the GC×GC were present at single digit µg/L levels. A complete list of unique TICs from this study and additional details about the GC×GC-MS analysis are presented in Mohler et al. (in press). DOI: 10.1021/es401706m.

Table 4
Results of Commercial Lab Analyses

Site and Well	TPHd/DRO		Field Readings		Bio Parameters			GC-MS Quant		GC-MS Library Search					
	TPHd	TPHd with SGC	ORP	DO	Nitrate	Iron +2	Sulfate	Methane	Detected Target Polars	K	Ph	Ald	Alc	Acids/Est	# Unique TICs
<i>Site 1</i>															
MW-26A	4400 p	690 p (0%)	-163/-161	1.2/1.2	<0.5ht	1.6	59	1.9	11 µg/L dodecanoic acid; all others ND	0	0	0	0	0	3
MW-5A	2100 p	2000 p (1%)	-99/-97	0.7/0.6	<0.5ht	0.2	115	<0.015	All ND	0	0	0	0	0	3
MW-100/5A	2000 p	540 p (0%)			<0.5ht	0.2	106	<0.015	All ND	0	0	0	0	0	6
MW-50A	1300	<98	-94/-57	1.5/1.3	<0.5ht	2	184	0.053	All ND	0	0	0	0	0	2
MW-71A	380	<100	30/23	1.3/1.3	5	<0.1	425	<0.015	All ND	0	0	0	0	0	1
<i>Site 2</i>															
MW-6	27,000 p	20,000 p	86/103	0.9/1.1	<0.5	4	10	0.71	All ND	0	1	0	0	0	1
MW-8	1200	<98	93/138	1.0/1.2	2.1	<0.1	34.6	0.025	All ND	0	0	0	0	0	1
MW-100/8	1100	<98			2	<0.1	33.5	0.022	All ND	0	0	0	0	0	1
MW-7	98	<100	93/128	0.8/1.1	2	<0.1	22.8	<0.015	All ND	0	0	0	0	0	1
<i>Site 3</i>															
MW-8	2600	410 (0%) ?	-5/13	1.0/1.4	1.4	27.8	19.2	2.9	All ND	0	0	0	0	0	4
MW-21	1000	<97	84/63	1.2/1.5	<0.5	6.8	5.8	3.1	All ND	0	0	0	0	0	3
MW-100/21	1100	<97			1.6	6.2	20.5	3.2	All ND	0	0	0	0	0	3
<i>Site 4</i>															
MW-3	3200	<96	184 pre	1.6 pre	2.2	4.6	73.7	5.1	All ND	0	0	0	0	0	7
MW-100/3	2900	<120							All ND	0	0	0	0	0	2
MW-41	3300	<96	-24 pre	1.7 pre	5.9	11.7	19.9	1.2	All ND	0	0	0	0	0	1
MW-26	210	<100	108 pre	1.2 pre	16.1	0.17	31.5	0.021	All ND	0	0	0	0	0	2
MW-31	470	<100	93 pre	1.9 pre	1.3	10.5	94.9	0.74	All ND	0	0	0	0	0	2
<i>Site 5</i>															
MW-21	8100	840 (0.7%)	163 pre	1.4 pre	<0.5	41.1	<5.0	3.5	All ND	3	0	1	0	0	4
MW-22	6000	800 (1.2%)	142 pre	1.2 pre	0.6	23.2	20	4.8	All ND	2	3	0	0	0	3
MW-100/22	6300	840 (1.3%)			<0.5	22.1	16.9	5.5	All ND	2	1	0	0	0	3
MW-5	1700	150 (0%) ?	107 pre	1.2 pre	<0.5	<0.1	41	<0.015	All ND	0	0	0	0	0	2
MW-13	1100	380 (0%) ?	159 pre	1.3 pre	<0.5	2.2	1660	<0.015	All ND	0	0	0	0	0	2

Notes: Shaded rows are source area wells; MW-100/xx is blind field duplicate of well xx. TPHd/DRO in µg/L using Method 8015 (C10 to C28); SGC= Method 3630C (capric acid recovery 0% unless stated); p= Product (nondissolved) component present. Oxidation Reduction Potential (ORP) in mV; Dissolved Oxygen (DO), Nitrate, Ferrous Iron, Sulfate, and Methane in µg/L. Detected Target Polars = 57 compounds analyzed using GC-MS (Mod Method 8270), quantified against standards, in µg/L; analysis performed on a DCM (no SGC) extract and the methanol eluate off the SG column. All ND = all compounds non-detect for both the DCM extract and the methanol eluate; detection limits mostly 10 µg/L. K = ketones; Ph = phenols; Ald = aldehydes; Alc = alcohols; Acids/Est = acids and esters. Library Search = Open scan GC-MS; attempt to identify top 40 peaks; number of unique individual tentatively identified compounds (TICs) in each polar family; results for DCM extracts and methanol eluate are combined. pre = pre-purge measurement; other shown as pre-purge/post-purge. ?= SGC at Site 5 MW-5 and MW-13 did not completely remove high-boiling polars UCM. ?= SGC at Site 3 MW-8 did not completely remove high-boiling polars UCM.

Table 5

Summary of GC×GC-MS Results: Average per Well % of TICs by Polar Families and by Expected Human Chronic Oral Toxicity

Site	Avg TPHd wo/w SGC µg/L	Total # TICs #	Polar Chemical Families					Expected Chronic Oral Toxicity		
			Acids	Alcohols	Phenols	Ketones	Aldehydes	Low to Moderate		
			%	%	%	%	%	Low	Moderate	Moderate
<i>Total Results—All Samples and All Sites</i>										
Site 1	—	207	50	23	2	22	3	82	17	1
Site 2	—	213	56	32	1	9	2	92	7	1
Site 3	—	80	43	23	0	28	6	80	20	0
Site 4	—	181	47	24	3	21	5	90	10	0
Site 5	—	772	34	25	5	31	4	78	17	5
Average % all sites			46	25	2	22	4	84	14	1
<i>Source Zone Samples</i>										
Site 1	2800 p / 1100 p	173	36	30	2	28	3	78	20	2
Site 2	27,000 p / 20,000 p	173	25	31	3	32	8	72	25	3
Site 3	1600 / 200	80	43	23	0	28	6	80	20	0
Site 4	3100 / <100	153	31	29	0	32	8	86	14	0
Site 5	6800/830 nc	737	13	26	8	45	7	65	26	8
Average % source area			30	28	3	33	6	76	21	3
<i>Downgradient Samples</i>										
Site 1	840 / <100	34	70	13	2	13	2	88	12	0
Site 2	800 / <100	40	66	32	0	2	0	98	2	0
Site 4	340 / <100	28	71	16	8	6	0	98	2	0
Site 5	1400 / 270 nc	35	65	23	0	12	0	97	3	0
Average % down-gradient area			68	21	2	8	1	95	5	0

Notes: TICs = tentatively identified compounds; Avg TPHd wo/w SGC = average concentration of total petroleum hydrocarbons as diesel (TPHd) without silica gel cleanup (SGC) and with SGC. “Without SGC” includes hydrocarbons and polars, “with SGC” represents hydrocarbons only; µg/L = micrograms per liter; # = number; % = percent; -- = not calculated; p = sample contained a product (nondissolved) component; nc = SGC was not complete; both wells at Site 3 were classified as source area; Low, Low to Moderate, and Moderate toxicity are defined in the body of the article; “Phenols” in downgradient samples were phenol only and no alkyl phenols. Alkyl phenols were identified only in source area samples.

The GC×GC-MS analysis resulted in a greatly increased number of polar metabolite TICs for each site, ranging from 80 (Site 3) to 772 (Site 5). The number of unique polar metabolite TICs in a single well ranged from 5 to 310. The greatly increased number of TICs for the GC×GC-MS as compared to the GC-MS confirms that two-dimensional chromatography is necessary to resolve individual compounds in these complex mixtures of polar metabolites. The highest number of polar metabolite TICs (and highest concentration of polars measured as TPHd) was present in source-area samples at each site, with significantly fewer TICs (and very low concentrations of polars as TPHd) in downgradient samples at each site.

Summaries of the average percentage of TICs in each of the polar metabolite families for each site, and separately for the source and downgradient areas at each site, are shown in Table 5. For this study, average percentage calculations are always based on the percentage for each individual well at

each site, and not on the total number of TICs for the site as a whole, so that results for the downgradient samples (with fewer TICs) are weighted equally with source-area samples. The average TPHd concentration without and with SGC for each area at each site is also shown in Table 5 to provide context regarding the “bulk” concentration of polar compounds in each area.

The site-wide results for all five sites were similar and indicate that an average of 46% of the polar metabolite TICs were acids/esters, 25% were alcohols, 2% were phenols, 22% were ketones, and 4% were aldehydes (Table 5, Figure 2). All five sites also showed that there is a marked difference in the distribution of the identified polar metabolite families between source-area and downgradient samples at each site. In source-area samples, the acids/esters (average of 30% of the polar metabolite TICs), alcohols (28%), and ketones (33%) are approximately equally distributed, with far fewer phenols (3%, including alkyl phenols) or aldehydes (6%). In

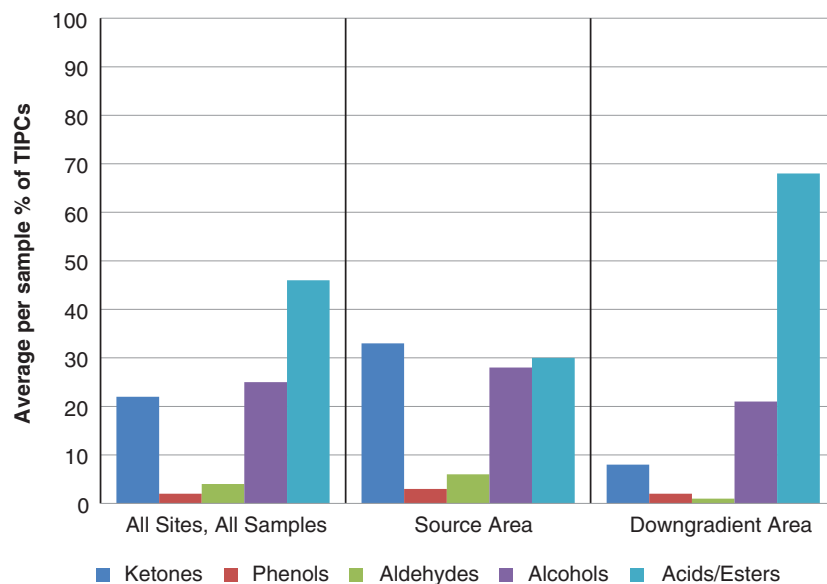


Figure 2. Summary of GC×GC-MS results for polar families: distributions overall and in source and downgradient areas.

downgradient samples, the acids/esters dominate (average of 68% of the polar metabolite TICs), with fewer alcohols (21%), and far fewer ketones (8%), phenols (2%, all as phenol and no alkyl phenols), and aldehydes (1%). Although the GC×GC results are not quantitative, the relative response factors show that it is reasonable to compare trends for each family and class within samples in the C8 to C12 carbon range.

A summary of the average percentage of identified structural classes in the source and downgradient areas for all five sites combined is shown in Table 6. All 22 expected structural classes were tentatively identified. The most frequently identified structure was alkyl acids/esters, which averaged about 21% of the polar metabolite TICs in the source area and about 56% of the polar metabolite TICs in the downgradient-area samples. The results show that, when identified, the more complex bicyclic and polycyclic aromatic structures are primarily in source-area samples, as would be expected due to their proximity to the residual hydrocarbon source, with less complex structures predominating in downgradient samples. Alkyl phenols were identified in only five samples, all of which were in source areas and two of which contained a nondissolved product component.

Because the relative response factors for each polar metabolite family were reasonably similar, the GC×GC-MS results were also evaluated by reviewing each sample to determine the polar families that were represented in the top 5, 10, and/or 20 peaks by peak area response. This showed that for each sample the acids and/or alcohols were typically a higher percentage of the highest peak area response than their percentage based on the number of TICs. Conversely, the ketones, aldehydes, and phenols were typically a smaller percentage of the highest peak area response than their percentage based on the number of TICs. This means that the results as presented in this paper based on numbers of TICs may underestimate the proportion of acids and/or alcohols, and may overestimate the proportion of ketones, aldehydes, and phenols, actually present in each sample.

Toxicity Evaluation

A summary of the average percentage of the total number of polar metabolite TICs in each toxicity ranking (Low, Low to Moderate, and Moderate) for each site, and separately for source and downgradient areas at each site, is shown in Table 5. For all five sites combined, an average of 84% of the polar metabolite TICs are ranked as “Low” toxicity, 14% as “Low to Moderate” toxicity, and only 1% as “Moderate” toxicity. In source-area samples, the average percentages are 76% “Low” toxicity, 21% “Low to Moderate” toxicity, and 3% “Moderate” toxicity. In downgradient samples, the profile shifts toward a lower toxicity, with average percentages of 95% “Low” toxicity, 5% “Low to Moderate” toxicity, and 0% “Moderate” toxicity. The increase in the average percentage of “Low” toxicity compounds and the decrease in the “Low to Moderate” and “Moderate” toxicity compounds in the downgradient samples is due to the dominance of acids/esters, and the virtual lack of aromatic/polycyclic aromatic ketones, alkyl phenols, and aldehydes in downgradient samples (Table 6).

Conclusions

The purposes of this study were to (1) identify as well as possible the polar compounds in the DCM extracts of groundwater samples from five sites with biodegrading fuel sources that are quantified as TPHd unless a SGC is used to separate the polars from hydrocarbons, and (2) estimate the potential chronic human toxicity of the identified polar compounds. The GC×GC-MS analysis provided detail not previously available for actual groundwater samples, and documented that the vast majority of polar compounds identified in the groundwater sample extracts were oxygen-containing metabolites of biodegradation. The mixture of identified polar metabolites is composed of organic acids/esters, with variable alcohols and ketones, and very few phenols and aldehydes. The analytical results were similar among the five sites. The mixture of identified polar

Table 6
GC×GC-MS Results—Avg% for Each Structural Class in Source and Downgradient Areas

Polar Family	Specific Structural Class	Expected Chronic Oral Toxicity to Humans	Source Area Samples	Downgradient Samples
			Avg % per Sample	Avg % per Sample
Alcohols (and diols)	Alkyl alcohols	Low	13	11
	Cycloalkyl alcohols	Low	10	5
	Bicyclic alkyl alcohols	Low	2	6
	Aromatic alcohols	Low	3	<1
	Polycyclic aromatic alcohols	Low to Moderate	<1	0
Acids (and esters)	Alkyl acids	Low	21	56
	Cycloalkyl acids	Low	2	<1
	Bicyclic alkyl acids	Low	2	0
	Aromatic acids	Low	3	12
	Polycyclic aromatic acids	Low to Moderate	<1	0
Ketones	Alkyl ketones	Low to Moderate	6	3
	Cycloalkyl ketones	Low	9	2
	Bicyclic alkyl ketones	Low	9	2
	Aromatic ketones	Low to Moderate	8	<1
	Polycyclic aromatic ketones	Low to Moderate	<1	0
Aldehydes	Alkyl aldehydes	Low to Moderate	4	<1
	Cycloalkyl aldehydes	Low to Moderate	<1	0
	Bicyclic alkyl aldehydes	Low to Moderate	<1	0
	Aromatic aldehydes	Low to Moderate	2	0
	Polycyclic aromatic aldehydes	Low to Moderate	<1	0
Phenols	Alkyl phenols	Moderate	3	0
	Phenol	Low	<1	2

Average % per sample is based on the % by number of TICs for each well.

metabolites in the source area samples had approximately equal average percentages of organic acids/esters, alcohols, and ketones, which reflects the ongoing sequential oxidation reactions proximal to the residual hydrocarbon molecules. The mixture of identified polar metabolites in the downgradient area samples was dominated by organic acids/esters. The observed spatial trend in the relative proportions of the polar families, combined with the predominant simpler structures and decreasing bulk concentrations of polar compounds (measured as TPHd) seen in downgradient samples, documents the continued biodegradation of the polar metabolites themselves and their ultimate natural attenuation with migration away from the residual hydrocarbon in the source area. The oxidation of the various polar families to small organic acids, and their ultimate transformation to carbon dioxide and water, is consistent with known metabolic pathways.

An RfD-based toxicity ranking system that is consistent with systems used by USEPA and the United Nations was developed and applied to each of the identified polar metabolite structural classes. The results from this study show that the vast majority of the hundreds of polar metabo-

lites that were identified using GC×GC-MS in groundwater sample extracts from these five biodegrading fuel sites are in structural classes of “Low” toxicity hazard to humans. These results indicate that the mixtures of polar metabolites identified in groundwater extracts at these five sites are unlikely to present a significant human health risk, assuming that the affected groundwater were to be consumed as drinking water.

The results from this study also show (and confirm results from previous studies) that the organics in groundwater quantified as TPHd at these five sites were primarily polar metabolites and not dissolved hydrocarbons. Therefore, a SGC is necessary if groundwater sample TPHd results are to be compared to hydrocarbon-based regulatory criteria.

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