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Toxicity Assessment of Groundwater Contaminated by Petroleum Hydrocarbons at a Well-Characterized, Aged, Crude Oil Release Site

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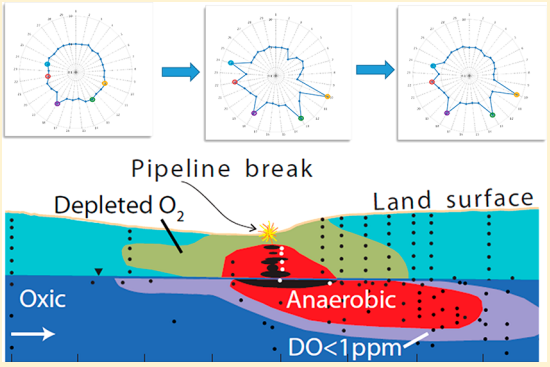
Supporting Information

ABSTRACT: Management of petroleum-impacted waters by monitored natural attenuation requires an understanding of the toxicology of both the original compounds released and the transformation products formed during natural breakdown. Here, we report data from a groundwater plume consisting of a mixture of crude oil compounds and transformation products in an effort to bridge the gap between groundwater quality information and potential biological effects of human exposures. Groundwater samples were characterized for redox processes, concentrations of nonvolatile dissolved organic carbon (NVDOC) and total petroleum hydrocarbons in the diesel range, as well as for activation of human nuclear receptors (hNR) and toxicologically relevant transcriptional pathways. Results show upregulation of several biological pathways, including peroxisome proliferator-activated receptor gamma and alpha, estrogen receptor alpha, and pregnane X receptor (PXR) with higher levels of hNR activity observed in more contaminated samples. Our study of affected groundwater contaminated by a crude-oil release 39 years ago shows these types of waters may have the potential to cause adverse impacts on development, endocrine, and liver functioning in exposed populations. Additionally, positive trends in activation of some of the molecular targets (e.g., PXR) with increasing NVDOC concentrations (including polar transformation products) demonstrate the importance of improving our understanding of the toxicity associated with the unknown transformation products present in hydrocarbon-impacted waters. Our results begin to provide insight into the potential toxicity of petroleum-impacted waters, which is particularly timely given the ubiquitous nature of waters impacted by petroleum contamination not only recently but also in the past and the need to protect drinking-water quality.



INTRODUCTION

Comprehensive characterization of the exposure and effects associated with petroleum- and fuel-impacted waters is essential for protection of human health and ecosystem integrity. At sites where water is contaminated with petroleum hydrocarbons, the analyses required by the U.S. Environmental Protection Agency include dissolved total petroleum hydrocarbons in the gasoline, diesel, or oil range (TPHg, TPHd, or TPHo). At old spill sites, much of the dissolved organic matter consist of polar compounds from the partial degradation of hydrocarbons in situ.[1] Recent results based on nonvolatile dissolved organic carbon (NVDOC) analyses show that polar transformation products quantified with TPHd analyses represent only a fraction of the polar transformation products present at a refined fuel site[2] and a crude-oil spill site.[3] Due to the vast number of residual oil and fuel spills that remain in the subsurface[4] characterization of the concentrations, toxicity, and natural attenuation of transformation products (metabolites) is essential to protect water quality.

As groundwater impacted by crude oil or petroleum hydrocarbon fuel spills ages and undergoes natural attenuation, both the original compounds and the more soluble breakdown products migrate away from the spill, forming a groundwater plume. In the 1990s, considerable evidence suggested that the spatial extent of such plumes was limited (i.e., plume length of indicator compounds such as benzene would "stabilize" then shrink) due to natural attenuation processes, principally biodegradation by in situ bacterial populations, at rates controlled by availability of electron acceptors such as oxygen, nitrate, iron oxyhydroxides, and sulfate.[5] However, recent studies suggest that this assumption may not be sufficiently protective of water resources. For example, a study of 10 closed hydrocarbon sites in Wisconsin found that benzene concen-

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Table 1. Site Characteristics—Biogeochemistry and Chemistry

well number	310B	956	533E	531A	9315B
sample type	reference	spray zone	below oil body	plume	plume
distance from center of the oil body (m)	−200.5	−125	38.8	67.6	101.6
biogeochemical characterization	suboxic	aerobic	methanogenic	methanogenic	iron reducing
nonvolatile dissolved organic carbon (mg/L of C)	1.66	14.7	25.9	16.3	15.2
total petroleum hydrocarbons diesel range (mg/L)	BDL	0.92	6.6	3.5	2.8
CH_4 (mg/L)	0.07	<0.01	7.93	3.16	0.63
arsenic (μg/L)	3.60	0.620	57.0	50.1	13.7

trations exceeded those measured at the time of closure.[6] In addition, because the breakdown products of petroleum hydrocarbons can have lower volatility and greater polarity (and thus greater solubility), their fate and transport properties are quite different than those of the parent compounds[7] and would be expected to form different plumes lengths and create different toxicity profiles upon exposure. Thus, comprehensive studies of older plumes are critical to understand the potential effects of exposure to petroleum-impacted waters.

Here, we report data from groundwater collected from The National Crude Oil Spill Fate and Natural Attenuation Research Site near Bemidji, MN, U.S.A. This site is ideal for investigating the toxicity of waters impacted by both petroleum hydrocarbons and their breakdown products, as the hydrological, microbiological, and geochemical controls on biodegradation and contaminant transport have been studied for nearly 40 years https://mn.water.usgs.gov/projects/bemidji/. Prior work has shown that the 50 ug/L contour of benzene is relatively stable, while the plume of breakdown products, as measured as nonvolatile dissolved organic carbon (NVDOC), extends well beyond the benzene plume. Modeling indicates that concentrations of NVDOC decrease under anaerobic conditions at a first-order rate of 0.13% per day,[8] but the position of the 5 mg/L (as carbon) contour has expanded from 125 m downgradient in 1988 to over 200 m in 2010.[3] In addition, concentrations of NVDOC from the crude oil are 10−20 times higher than the concentrations of benzene and 3−23 times higher than TPHd.[3] Thus, the required analyses do not include many of the organic compounds migrating in groundwater from residual petroleum hydrocarbon sources. In addition, the possible biological effects of exposure to waters that contain a complex mixture of known and unknown transformation products are unknown.

Risk assessment of petroleum- and fuel-impacted waters has mostly focused on the evaluation of the acute and baseline toxicity (a.k.a. narcosis—a nonspecific toxicity due to the disruption of the lipid membrane[9,10]). Podgorski et al. found a positive correlation between acute toxicity and NVDOC data[11] on groundwater samples collected from this study site. Emerging evidence suggests that petroleum-impacted waters also have a potential to initiate adverse effects by specific interactions with molecular targets (e.g., aryl hydrocarbon, estrogen and androgen receptor[12−14]). A more complete characterization of specific mechanisms of toxicity of petroleum-impacted waters is needed. Given the complex composition of dissolved organic matter in aged plumes (ca. 15 000 chemicals at the site of the present study[15]) and a lack of knowledge of identities and toxicity of the individual chemicals, the characterization of specific toxicity based on chemical composition alone is not feasible. Bioeffects-based assays can be used instead, as they allow for time- and cost-effective screening of activation of specific molecular targets by complex mixtures.[16]

Here we present results of comprehensive in vitro biological activity screening combined with measurements of TPHd and NVDOC to identify potential mechanisms of toxicity of a mixture of crude-oil compounds and their transformation products. Results indicate the potential of petroleum-impacted waters to activate human nuclear receptors (hNR) and several transcription factors, including those for which disruption may lead to developmental toxicity, endocrine disruption, and metabolic dysfunction.

MATERIALS AND METHODS

Study Site. This study was conducted at the National Crude Oil Spill Fate and Natural Attenuation Research Site near Bemidji, MN, U.S.A., because it offers an opportunity to characterize toxicity in the context of monitored natural attenuation (MNA). The site was contaminated in 1979 when a buried pipeline ruptured spraying an estimated 1.7 million liters of light crude oil on the land surface. Sprayed oil coated the surface soil (referred to here as the spray zone) and flowed toward local depressions where it infiltrated the aquifer forming three subsurface oil bodies (Figure S1 of the Supporting Information, SI). Groundwater is contaminated below the spray zone and downgradient from the oil bodies. The spray zone and contaminant plume movement,[8] redox zones,[17] biogeochemistry, and microbiology are well characterized.[18]

In August 2016, water samples were collected from 30 groundwater locations at the site, representing a range of redox conditions and organic chemical composition.[11] A location upgradient from the contaminant plume was used as a reference sample for the uncontaminated aquifer.[17] Water samples were characterized for biogeochemical properties, including dominant redox zonation and organic chemistry. A subset of samples collected from five wells, representing the different biogeochemical zones, (310B, 956, 533E, 531A, 9315B; Table 1) were also screened for biological activity using a battery of in vitro assays.

Water Chemistry. Groundwater samples were collected after the well was purged to at least three times the water in the well casing and field parameters (temperature, dissolved oxygen, specific conductance, and pH) were stabilized. All water chemistry samples were stored on wet ice in the field and then refrigerated at 4 °C in the laboratory until analyzed. Samples for NVDOC were filtered through 0.20-μm Supor in-line filters into baked glass bottles, preserved with hydrochloric acid to a pH of <2, and analyzed using a Shimadzu TOC Vcsn analyzer (Shimadzu Corporation).

Samples for total petroleum hydrocarbons in the diesel range (TPHd) were collected into 1-L amber bottles with Teflon lined caps and preserved with 5 mL of 50% HCl at the time of collection; these samples were extracted within 7 days of sample collection. The samples were analyzed by Pace Analytical, St. Paul, MN, using the Wisconsin modified DRO method.[19] Briefly, organic constituents were extracted with hexane,

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analyzed with a gas chromatograph and a flame ionization detector (FID), and quantified based on a diesel component standard. The reporting limit was 0.1−0.54 mg/L.

Samples for methane (CH_4) concentrations were collected in Glaspak syringes connected directly to the sample-pump outlet and transferred into 25 mL serum bottles containing TSP (trisodium phosphate dodecahydrate). Dissolved CH_4 concentrations were measured by headspace analysis using a 5890 Series II HP Gas Chromatograph split/splitless inlet FID with a fused silica capillary column.

Samples for total dissolved arsenic (As_T) were filtered in-line through 0.2 μm Nuclepore membranes and preserved to pH < 2 with double distilled nitric acid. Dissolved As_T was analyzed using a PerkinElmer ELAN 9000 Inductively Coupled Plasma Mass Spectrometry (ICP-MS), with a detection limit for dissolved As_T of 0.1 μg/L, and reported as total concentration. Arsenic species were not identified, but Cozzarelli and colleagues[17] showed As(III) accounts for 80−100% of As species present in the anoxic groundwater.

Biological Activity. Water samples (500 mL) from 5 sites, shown in Table 1, were kept on dry ice in the field and frozen at −20 °C until processed. Samples were filtered using a GF/F filter (1.0 μm). Filtrates were concentrated using OASIS HLB 5 cm^3 200 mg cartridges (Waters, Milford, MA), eluted with 50:50 methanol: dichloromethane, and brought to dryness under nitrogen gas. Once dry, each sample was suspended in 0.5 mL dimethyl sulfoxide (DMSO). This preparation method removed the volatile fraction, including benzene, toluene, ethylbenzene and xylenes (BTEX), and is expected to remove the inorganic arsenic.[20] Each sample was tested in triplicate at three concentrations including in situ concentration (1×) and concentrated (3× and 10×).

Commercially available, well-characterized TRANS- and CIS-FACTORIAL assays (Attagene Inc. Morrisville, NC) were used[21] to assess biological activities of water samples. TRANS-FACTORIAL can measure water-sample activities against 48 human nuclear receptors (hNR). CIS-FACTORIAL measures the effects on the activity of more than 40 toxicologically relevant transcriptional pathways. (SI Tables S1 and S2). FACTORIAL assays use a library of reporter constructs ("reporter transcription units"—RTUs) similar to conventional reporter gene constructs where a transcription factor (TF) responsive promoter is linked to a downstream reporter sequence. FACTORIAL assays do not rely on translation of RTU transcripts into proteins; the reporter transcript abundance is measured (total RNA is isolated, reverse-transcribed, amplified, labeled, and quantified by capillary electrophoresis).[21] The methodology is distinct from conventional approaches, because the RTUs have identical reporter sequences. Distinction between reporter transcripts is achieved by tagging of the reporter sequences with "processing tags" that identify a unique cleavage position for each of the reporter cDNAs. The main difference between the two types of assays is that CIS- measures activities of endogenous TFs, whereas the TRANS- evaluates changes in activities of exogenous, chimeric NR-Gal4 proteins.[21] Because the HepG2 cell line does not express all nuclear receptors the TRANS assay is used to complement the CIS assay's hNR suite.

Molecular target activation data for individual samples at 10× concentration were expressed relative to induction by 10 uL/L DMSO (solvent control), log_2 transformed and median centered. Euclidean distance average linkage hierarchical clustering (Heatmapper software[22]) was conducted on the transformed data (SI Figure S2).

RESULTS AND DISCUSSION

Water Chemistry. Concentrations of NVDOC and TPHd (Figure 1) were highest near the oil source and decreased with distance in the direction of groundwater flow. NVDOC concentrations were greater than three times the TPHd concentrations near the source and over 20 times higher beyond 150 m, consistent with earlier results that NVDOC analyses capture partial transformation products not measured in the TPHd analyses.[3]

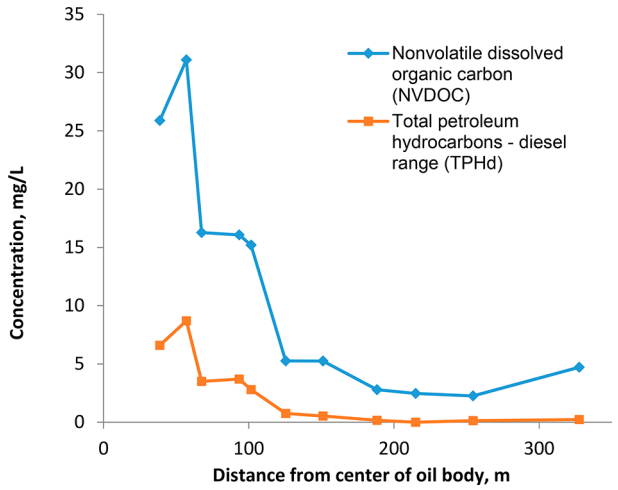

Figure 1. Concentrations of nonvolatile dissolved organic carbon (NVDOC) and total petroleum hydrocarbons in the diesel range (TPHd) measured August, 2016, along the centerline of the dissolved plume at the study site near Bemidji, Minnesota, U.S.A. Well locations and oil spill source location shown in SI Figure S1.

Locations, redox conditions, and chemical data for the five wells screened for biological activity are shown in Table 1, including a background, suboxic well with naturally occurring organic matter (310B), a well below the subsurface oil source (533E), two wells in the plume (531A and 9315B), and a well below the spray zone (956). Contaminants within the spray zone were exposed to sunlight and oxygen and thus subjected to photooxidation as well as aerobic biodegradation. Dissolved arsenic (and other metals) are released into the groundwater system when reduction (and dissolution) of naturally occurring iron oxide minerals coupled to the oxidation of organic matter occurs.[23] The generation of dissolved CH_4 is an additional effect of the hydrocarbon biodegradation in the most contaminated portions under the oil bodies.[18]

Biological Activity. Activation of several human nuclear receptors (hNR) was detected (Figure 2A). The background sample (310B) only activated the pregnane X receptor (PXR). In the spray-zone sample (956), peroxisome proliferator-activated receptor gamma (PPARg), estrogen receptor alpha (ERa) and PXR were upregulated more than 2-fold compared to controls. The most highly activated hNRs in the water collected from beneath the oil body (well 533E) included ERa, ERb, PXR, PPARg, PPARa, and retinoic acid receptor beta (RXRb). Those same nuclear receptors responded to the waters collected from downgradient within the contaminant plume (531A and 9315B), but typically with lower magnitude (Figure 2A). CIS-FACTORIAL activation patterns were similar—the background

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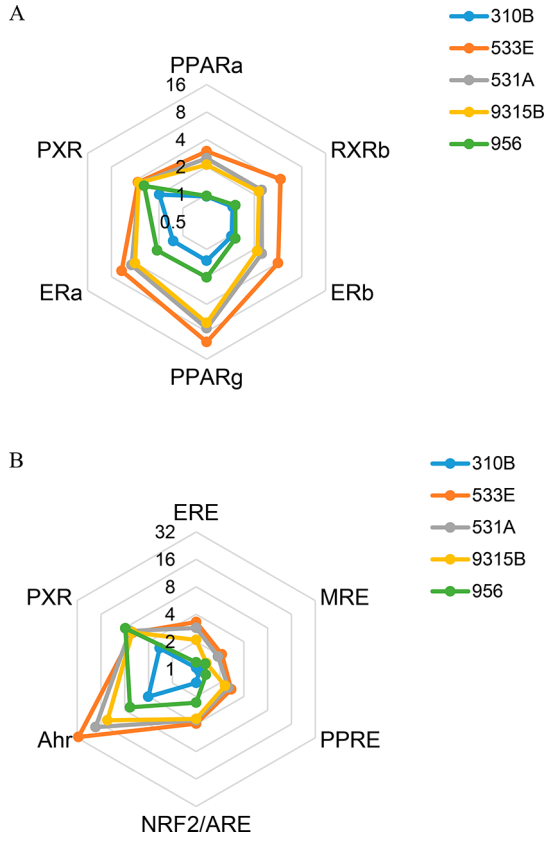

Figure 2. TRANS-FACTORIAL (A) and CIS-FACTORIAL (B) molecular target activation (fold change relative to DMSO control, *y* axis is log_2-scaled) by environmental sample concentrates (10×). Only molecular targets up/downregulated more than 2-fold in at least one of the wells are shown. PPARa: Peroxisome proliferator-activated receptor-alpha, PPARg: Peroxisome proliferator-activated receptor-gamma, PXR: Pregnane X receptor, ERa: Estrogen receptor-alpha, ERb: Estrogen nuclear receptor-beta, RXRb: Retinoid X receptor-beta, PXR: The pregnane X receptor (PXR), Xenobiotic Pathway, Ahr: The Aryl hydrocarbon receptor (AhR)/Xenobiotic Response, ERE: Estrogen Receptor (ER) pathway, MRE: Metal regulatory transcription factor 1 (MTF-1), PPRE: Peroxisome proliferator activating receptor (PPAR)a, d, g, NRF2/ARE: Antioxidant Response Element (ARE)-binding Nuclear factor (erythroid-derived 2)-like 2 (NRF2).

sample (310B) only mildly upregulated the PXR and aryl hydrocarbon receptor (AhR) pathways, whereas sample 533E, collected from beneath the oil body, activated the highest number of targets, and typically with the highest magnitude (Figure 2B).

Hierarchical clustering of biological activity data (SI Figure S2) revealed that the well below the main oil body (533E) was distinct from the others. Waters collected from downgradient, within the plume, clustered together (531A and 9315B). Similarly, background (310B) and spray-impacted wells (956) clustered together. Overall, clustering indicated distinct transcriptional activation profiles for samples collected beneath the oil body versus within the plume versus the background and spray zone wells.

A small number of molecular targets had a strong influence on the clustering of sample 533E (water beneath the oil body) away from the activity profiles of other samples. These included receptors PPARa, ERb, PXR, and pathways associated with peroxisome proliferator activating receptor (PPRE), estrogen receptor (ERE), and nuclear factor erythroid 2-related factor antioxidant (NRF2ARE) (SI Figure S2). Several of those targets are related (i.e., PPARa and PPRE, ER and ERE) and were activated in the two distinct sets of assays (Figure 2). The congruency between CIS- and TRANS- data provides strong evidence that these molecular targets and associated pathways can be activated by the oil-body derived contaminants. Notably, the AhR clustered away from all other genes and was highly upregulated in the set of samples collected below the oil body and within the plume.

Only six targets per assay type (CIS vs TRANS) were up/downregulated more than 2-fold in at least one of the analyzed groundwater samples. Because of a paucity of comprehensive transcriptomic and/or in vitro TF/hNR data (especially for mammalian models and weathered crude oils) it is difficult to know whether responses from the broader set of hNRs and/or TFs should have been observed in this study or could be expected at other similarly impacted sites. The present study is the first to comprehensively screen biological activity of groundwater from an aged crude oil release site. Below, we provide a brief review of the literature, including mining databases (i.e., Toxcast) to determine whether biological responses observed in the present study have been documented by other studies that investigated the toxicity of petroleum-impacted waters.

Activation of the AhR and ERb observed in the present study is consistent with the findings generated by focused screenings of four crude oils and seven refined fuel products for hNR activity using conventional reporter gene assays. For example, 100% of the 11 tested samples activated ERb[24] and AhR.[12] The maximum potencies of these samples were found to be highly variable—from 40 to a million times lower than the potency of well-known AhR agonists benzo[a]pyrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin.[12] Both aromatic and polar fractions of the crude oil have been identified as sources of the AhR agonists.[25] AhR induction of CYP enzyme activities, particularly CYP1A1, through the aryl hydrocarbon receptor (AhR) in most vertebrate species is a well-documented response to planar and aromatic organic contaminants.[26] Temkin et al.[27] reported that crude oil did not activate PPARg in vitro, but other studies have shown that exposure to PAHs induced PPAR signaling pathways.[28] In vivo studies with larval fish exposed to weathered crude oil also indicated interaction with PPAR pathways, and noted extensive interaction with the thyroid receptor/RXR and liver X receptor/RXR toxicity pathways.[29] The present study also noted effects on RXRb which is known to form heterodimers with the thyroid (TR) and vitamin D (VDR) receptors. Because nonpermissive heterodimers (i.e., those RXRb forms with TR or VDR) can only be activated by the partner's ligand while RXR is silent, the activation of RXR by samples 531A, 9315B, and 956 indicates potential effects on the thyroid and vitamin D signaling.[30]

In contrast to the above studies of oil and refined fuels, there is a paucity of studies that characterize biological activity of the chemicals that comprise complex mixtures of partial transformation products found at attenuated, aged sites (ca. 15 000 chemicals at our study site[15]). Because 18 low-molecular-weight organic acids were quantified at this site in the past,[31] we used ToxCast data to determine their potential to exert biological activities in the Attagene assays utilized in the present study. Exposure to activity ratios (EARs) were calculated by dividing the concentration of each organic acid by its activity concentration at cutoff (ACC) in Attagene assay.[32] The ACC estimates the chemical concentration at which the activity cutoff

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is achieved within an assay of interest. Because of the size and complexity of the ToxCast data we used *toxEval 1.0.1* to calculate EARs. The results indicate that some of the organic acids, if present in the current samples, could have contributed to PXRE and NRF2-ARE activity in the CIS- assays, and GR and PPARa activity in the TRANS- assays. However, the EARs for the individual organic acids were very low (<0.0048) indicating that additive effects and/or additional chemicals should be considered. It is important to note that some of the TOX21 assays, unlike Attagene, indicated potential of the examined organic acids to agonize ERa (i.e., benzoic acid had EAR of 0.11). This indicates that using multiple suites of assays may be beneficial when conducting initial screenings, as the assays may vary in sensitivity and may be susceptible to interference associated with the complex nature of the sample. The approach identified in this study is suitable for screening of molecular targets and chemicals of interest, but follow-up effects directed analyses that use well-characterized assays, combined with novel, chemometric reduction approaches[25] should be conducted to identify groups of chemicals that might be the drivers of the biological activities.

In the present study, the activation of the molecular targets was benchmarked to well-understood chemicals (i.e., several positive controls were incorporated in the experiments). Maximum activities of the in situ (1X) samples relative to their respective positive controls were as follows: ERa—12% of 0.2 μM 17β-estradiol, PXR—97% of 10 μM rifamicin, PPARg—11% of 1 μM rosiglitazone, and AhR—21% of the 1uM 6-Formylindolo [3,2-*b*] carbazole response. Extrapolating these molecular level, in vitro effects to the in vivo effects is difficult because of the complexity and unknown chemical composition of the tested groundwater samples. Typically, researchers use reverse dosimetry to extrapolate bioactive concentrations for individual chemicals in in vitro test systems to the comparable doses for in vivo exposure to test species or to humans.[33] Furthermore, in vitro assays have limited metabolic activity; understanding of the metabolism is critical to estimating in vivo toxicity of chemicals. Finally, predicting in vivo adverse outcomes without dose- and temporally intensive data brings additional uncertainty; the exposure regimen utilized by our study may not be representative of the likely environmental exposure. Thus, we limit our discussion of human health risks to suggest the direction for future studies.

The effects observed at the molecular level do not necessarily indicate adverse outcomes at the organismal level. The activation of the NRF2ARE pathway was likely initiated to protect cells from oxidative stress-induced cell death.[34] Activation of PXR and AhR might be a sign of chemical exposure and metabolism. PXR is activated by many endogenous and exogenous chemicals and one of the primary targets of PXR activation is the induction of CYP3A4, a phase I oxidative enzyme that aids with metabolism of chemicals.[35] PXR has been shown to be one of the most sensitive targets that is activated in these assays,[36] which is consistent with its well-documented role in xenobiotic sensing and metabolism.[37] Thus, induction of PXR by all groundwater samples (including background) is not surprising, nor should be assumed to lead to adverse outcomes. Similarly, AhR is best known for its role in mediating metabolism of xenobiotics.[38] AhR is typically involved in, though not limited to, regulation of biological responses to aromatic hydrocarbons (PAHs) and dioxins.[13,39] Activation of the AhR by exogenous chemicals has been shown to cause a range of adverse effects in fish, especially during early life stages.[40] Nevertheless, it is important to note that both PXR and AhR also mediate a variety of endogenous, "normophysiological" biological processes.[38,41] For example, AhR signaling has been indicated in mediation of neurogenesis and neuronal cell development, hematopoiesis, cardyomyogenesis, and endocrine regulation.[38] PXR is thought to regulate hepatic glucose and lipid metabolism, and the activation of PXR has been shown to cause glucose intolerance and hepatic lipid accumulation.[41] Thus, we propose that the interaction of the groundwater samples with AhR and PXR remains of interest, and that the future studies should determine whether adverse effects on the above pathways modulated by AhR and PXR are observed in vivo.

Activation of ERs might have implications for human health; dysregulation of estrogen receptor mediated pathways has been associated with endocrine disruption.[42] The query of the Comparative Toxicogenomics Database (ctdbase.org) for estrogen receptor (NCBI ID 2099) disease associations indicated breast, lung, prostatic and liver neoplasms, oligospermia, male and female infertility, hepatocellular carcinoma, and neoplastic cell transformation (those were top 10 disease associations based on the direct evidence query; CTD inference score >113).

Interference with normal PPAR function by contaminants can lead to the alteration of lipid and glucose metabolism and cellular differentiation.[43] PPARs have been identified as modulators of metabolic syndrome, cardiovascular disease, immune impairment and have been associated with the increased risks of cancer.[44]

Relationship of Biological Activity to Water Chemistry. Figure 3 shows concentrations of TPHd and NVDOC versus the upregulation of AhR and PXR at in situ concentrations (1×). A positive relationship between TPHd and AhR is evident, with AhR increasing from 1.6 in the background to 15.4 fold-increase in the most contaminated portion of the plume, as TPHd increases from below detection in the background to 6.6 mg/L in the most contaminated

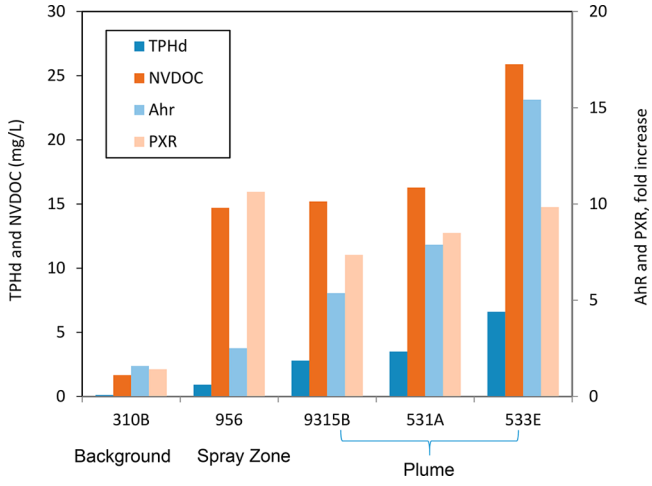

Figure 3. Concentrations of total petroleum hydrocarbons in the diesel range (TPHd), nonvolatile dissolved organic carbon (NVDOC) plotted together with upregulation of AhR and PXR at 1× concentration in the five tested wells. TPHd and AhR increase together. PXR is high at well 956 where TPHd is low but NVDOC is high indicating that the partial transformation products affect the response.

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portion of the plume. In contrast, TPHd and PXR appear less related because PXR activation is high in the spray zone where TPHd is low. PXR is more closely associated with NVDOC, suggesting that the upregulation of PXR may be related to transformation products created by photo-oxidation and aerobic biodegradation. It should be noted that bioeffects assays were conducted on sample extracts. Other potentially toxic components in the groundwater removed during extraction, such as arsenic and BTEX, may have additional biological effects. This underscores the importance of conducting additional studies that explore additive or antagonistic effects of multiple components in complex natural samples.

Although limited in scope, these results show a positive relationship between a complex mixture of nonvolatile hydrocarbons and transformation products, measured as NVDOC, and a subset of molecular targets, thus demonstrating a gap in our current understanding of the nature and effects of hydrocarbon-impacted waters. Though we recognize that correlation is not causation, the stronger relationship between some of the molecular targets (e.g., PXR targets) and NVDOC rather than TPHd demonstrates the importance of improving our understanding of the potential toxicity associated with the unknown transformation products present in hydrocarbon-impacted waters.

Our results expand the understanding of the potential toxicity of petroleum-impacted waters and demonstrate the need for additional data (not captured by current regulatory requirements for TPHd analyses) both in terms of the water chemistry (i.e., to include components of NVDOC not captured) as well as additional toxicological end points to evaluate the effectiveness of MNA as a remediation strategy for waters impacted by petroleum contamination. This need is particularly urgent given the ubiquitous nature of both new and residual source contamination and the need to protect drinking water quality.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b03657.

Site map, hierarchical clustering of the molecular target responses, list of molecular targets for TRANS assays, list of molecular targets for CIS assays, and TRANS-FACTORIAL and CIS-FACTORIAL molecular target activation by environmental sample concentrates (1×) (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Zemo, D. A.; O'Reilly, K. T.; Mohler, R. E.; Magaw, R. I.; Espino Devine, C.; Ahn, S.; Tiwary, A. K. Life cycle of petroleum biodegradation metabolite plumes, and implications for risk management at fuel release sites. *Integr. Environ. Assess. Manage.* **2017**, *13* (4), 714−727.

(2) Mackay, D.; Paradis, C.; Buscheck, T.; Daniels, E.; Hathaway, E.; de Sieyes, N.; Rasa, E.; Schmidt, R.; Peng, J. Methods to Estimate Source Zone Depletion of Fuel Releases by Groundwater Flow. *Groundwater Monit. Rem.* **2018**, *38*, 26−41.

(3) Bekins, B. A.; Cozzarelli, I. M.; Erickson, M. L.; Steenson, R. A.; Thorn, K. A. Crude Oil Metabolites in Groundwater at Two Spill Sites. *Groundwater* **2016**, *54* (5), 681−691.

(4) US EPA. Semiannual report of UST performance measures mid fiscal year 2018 (October 1, 2017-March 31, 2018); Office of Underground Storage Tanks: Washington, D.C. 20460,www.epa.ust 14 p.

(5) NRC. *In Situ Bioremediation*; National Academy Press: Washington, D. C., 1993; p 207.

(6) Evanson, T. A.; Pelayo, A. M.; Bahr, J. M. *Wisconsin Closure Protocol Study: A Retrospective Study of LUST Site Closures between 1999 and 2000*; Wisconsin Department of Natural Resources: PUB-RR-805: 2009; p 113.

(7) Zemo, D. A.; O'Reilly, K. T.; Mohler, R. E.; Tiwary, A. K.; Magaw, R. I.; Synowiec, K. A. Nature and Estimated Human Toxicity of Polar Metabolite Mixtures in Groundwater Quantified as TPHd/DRO at Biodegrading Fuel Release Sites. *Groundwater Monit. Rem.* **2013**, *33* (4), 44−56.

(8) Ng, G.-H. C.; Bekins, B. A.; Cozzarelli, I. M.; Baedecker, M. J.; Bennett, P. C.; Amos, R. T.; Herkelrath, W. N. Reactive transport modeling of geochemical controls on secondary water quality impacts at a crude oil spill site near Bemidji, MN. *Water Resour. Res.* **2015**, *51* (6), 4156−4183.

(9) Verhaar, H. J. M.; van Leeuwen, C. J.; Hermens, J. Classifying environmental pollutants. 1: Structure−activity relationships for prediction of aquatic toxicity. *Chemosphere* **1992**, *25*, 471−491.

(10) McGrath, J. A.; Parkerton, T. F.; Hellweger, F. L.; Di Toro, D. M. Validation of the narcosis target lipid model for petroleum products: Gasoline as a case study. *Environ. Toxicol. Chem.* **2005**, *24* (9), 2382−94.

(11) Podgorski, D. C.; Zito, P.; McGuire, J. T.; Martinovic-Weigelt, D.; Cozzarelli, I. M.; Bekins, B.; Spencer, R. G. M. Examining Natural Attenuation and Acute Toxicity of Petroleum-Derived Dissolved Organic Matter (DOMHC) with Optical Spectroscopy. *Environ. Sci. Technol.* **2018**, *52* (11), 6157−6166.

(12) Vrabie, C. M.; Jonker, M. T.; Murk, A. J. Specific in vitro toxicity of crude and refined petroleum products. 1. Aryl hydrocarbon receptor-mediated responses. *Environ. Toxicol. Chem.* **2009**, *28* (9), 1995−2003.

(13) Vrabie, C. M.; Sinnige, T. L.; Murk, A. J.; Jonker, M. T. Effect-Directed Assessment of the Bioaccumulation Potential and Chemical Nature of Ah Receptor Agonists in Crude and Refined Oils. *Environ. Sci. Technol.* **2012**, *46* (3), 1572−80.

(14) Jonker, M. T.; Candido, A.; Vrabie, C. M.; Scarlett, A. G.; Rowland, S. J. Synergistic androgenic effect of a petroleum product caused by the joint action of at least three different types of compounds. *Chemosphere* **2016**, *144*, 1142−7.

(15) Islam, A.; Ahmed, A.; Hur, M.; Thorn, K.; Kim, S. Molecular-level evidence provided by ultrahigh resolution mass spectrometry for oil-derived DOC in groundwater at Bemidji, Minnesota. *J. Hazard. Mater.* **2016**, *320*, 123−132.

DOI: 10.1021/acs.est.8b03657
Environ. Sci. Technol. 2018, 52, 12172−12178

(16) Schroeder, A. L.; Ankley, G. T.; Houck, K. A.; Villeneuve, D. L. Environmental surveillance and monitoring—The next frontiers for high-throughput toxicology. *Environ. Toxicol. Chem.* **2016**, *35* (3), 513−25.
(17) Cozzarelli, I. M.; Schreiber, M. E.; Erickson, M. L.; Ziegler, B. A. Arsenic Cycling in Hydrocarbon Plumes: Secondary Effects of Natural Attenuation. *Groundwater* **2016**, *54* (1), 35−45.
(18) Essaid, H. I.; Bekins, B. A.; Herkelrath, W. N.; Delin, G. N. Crude Oil at the Bemidji Site: 25 Years of Monitoring, Modeling, and Understanding. *Groundwater* **2011**, *49* (5), 706−726.
(19) *Modified DRO Method for determining diesel range organics*; PUBL-SW-141; Wisconsin Department of Natural Resources. 1995; https://dnr.wi.gov/regulations/labcert/documents/methods/DROSep95.pdf.
(20) Anthemidis, A. N.; Giakisikli, G.; Xidia, S.; Miró, M. On-line sorptive preconcentration platform incorporating a readily exchangeable Oasis HLB extraction micro-cartridge for trace cadmium and lead determination by flow injection−flame atomic absorption spectrometry. *Microchem. J.* **2011**, *98* (1), 66−71.
(21) Romanov, S.; Medvedev, A.; Gambarian, M.; Poltoratskaya, N.; Moeser, M.; Medvedeva, L.; Gambarian, M.; Diatchenko, L.; Makarov, S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. *Nat. Methods* **2008**, *5* (3), 253−60.
(22) Babicki, S.; Arndt, D.; Marcu, A.; Liang, Y.; Grant, J. R.; Maciejewski, A.; Wishart, D. S. Heatmapper: web-enabled heat mapping for all. *Nucleic Acids Res.* **2016**, *44* (W1), W147−53.
(23) Ziegler, B. A.; McGuire, J. T.; Cozzarelli, I. M. Rates of As and trace element mobilization caused by Fe-reduction in mixed BTEX-ethanol experimental plumes. *Environ. Sci. Technol.* **2015**, *49* (22), 13179−13189.
(24) Vrabie, C.; Candido, A.; Van den Berg, H.; Murk, A.; Van Duursen, M.; Jonker, M. Specific in vitro toxicity of crude and refined petroleum products: 3. Estrogenic responses in mammalian assays. *Environ. Toxicol. Chem.* **2011**, *30* (4), 973−980.
(25) Radović, J. R.; Thomas, K. V.; Parastar, H.; Díez, S.; Tauler, R.; Bayona, J. M. Chemometrics-assisted effect-directed analysis of crude and refined oil using comprehensive two-dimensional gas chromatography−time-of-flight mass spectrometry. *Environ. Sci. Technol.* **2014**, *48* (5), 3074−8.
(26) Arukwe, A.; Nordtug, T.; Kortner, T. M.; Mortensen, A. S.; Brakstad, O. G. Modulation of steroidogenesis and xenobiotic biotransformation responses in zebrafish (Danio rerio) exposed to water-soluble fraction of crude oil. *Environ. Res.* **2008**, *107* (3), 362−70.
(27) Temkin, A. M.; Bowers, R. R.; Magaletta, M. E.; Holshouser, S.; Maggi, A.; Ciana, P.; Guillette, L. J.; Bowden, J. A.; Kucklick, J. R.; Baatz, J. E.; Spyropoulos, D. D. Effects of Crude Oil/Dispersant Mixture and Dispersant Components on PPAR γ Activity in Vitro and in Vivo: Identification of Dioctyl Sodium Sulfosuccinate (DOSS; CAS# 577−11−7) as a Probable Obesogen. *Environ. Health Perspect.* **2016**, *124* (1), 112−9.
(28) Jung, K. H.; Kim, J. K.; Noh, J. H.; Eun, J. W.; Bae, H. J.; Kim, M. G.; Chang, Y. G.; Shen, Q.; Kim, S. J.; Kwon, S. H.; Park, W. S.; Lee, J. Y.; Nam, S. W. Characteristic molecular signature for the early detection and prediction of polycyclic aromatic hydrocarbons in rat liver. *Toxicol. Lett.* **2013**, *216* (1), 1−8.
(29) Xu, E. G.; Mager, E. M.; Grosell, M.; Hazard, E. S.; Hardiman, G.; Schlenk, D. Novel transcriptome assembly and comparative toxicity pathway analysis in mahi-mahi (Coryphaena hippurus) embryos and larvae exposed to Deepwater Horizon oil. *Sci. Rep.* **2017**, *7* (1), 44546.
(30) Evans, R. M.; Mangelsdorf, D. J. Nuclear receptors, RXR, and the big bang. *Cell* **2014**, *157* (1), 255−66.
(31) Cozzarelli, I. M.; Baedecker, M. J.; Eganhouse, R. P.; Goerlitz, D. F. The geochemical evolution of low-molecular-weight organic acids derived from the degradation of petroleum contaminants in groundwater. *Geochim. Cosmochim. Acta* **1994**, *58* (2), 863−77.
(32) Blackwell, B. R.; Ankley, G. T.; Corsi, S. R.; DeCicco, L. A.; Houck, K. A.; Judson, R. S.; Li, S.; Martin, M. T.; Murphy, E.; Schroeder, A. L.; Smith, E. R.; Swintek, J.; Villeneuve, D. L. An "EAR" on environmental surveillance and monitoring: A case study on the use of exposure−activity ratios (EARs) to prioritize sites, chemicals, and bioactivities of concern in Great Lakes waters. *Environ. Sci. Technol.* **2017**, *51* (15), 8713−24.
(33) Rotroff, D. M.; Wetmore, B. A.; Dix, D. J.; Ferguson, S. S.; Clewell, H. J.; Houck, K. A.; LeCluyse, E. L.; Andersen, M. E.; Judson, R. S.; Smith, C. M.; Sochaski, M. A. Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. *Toxicol. Sci.* **2010**, *117* (2), 348−358.
(34) Johnson, J. A.; Johnson, D. A.; Kraft, A. D.; Calkins, M. J.; Jakel, R. J.; Vargas, M. R.; Chen, P. C. The Nrf2−ARE pathway an indicator and modulator of oxidative stress in neurodegeneration. *Ann. N. Y. Acad. Sci.* **2008**, *1147* (1), 61−9.
(35) Willson, T. M.; Kliewer, S. A. PXR, CAR and drug metabolism. *Nat. Rev. Drug Discovery* **2002**, *1* (4), 259.
(36) Judson, R. S.; Martin, M. T.; Reif, D. M.; Houck, K. A.; Knudsen, T. B.; Rotroff, D. M.; Xia, M.; Sakamuru, S.; Huang, R.; Shinn, P.; Austin, C. P.; Kavlock, R. J.; Dix, D. J. Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. *Environ. Sci. Technol.* **2010**, *44* (15), 5979−5985.
(37) Banerjee, M.; Robbins, D.; Chen, T. Targeting xenobiotic receptors PXR and CAR in human diseases. *Drug Discovery Today* **2015**, *20* (5), 618−28.
(38) Nebert, D. W. Aryl hydrocarbon receptor (AHR): "pioneer member" of the basic-helix/loop/helix per-Arnt-sim (bHLH/PAS) family of "sensors" of foreign and endogenous signals. *Prog. Lipid Res.* **2017**, *67*, 38−57.
(39) Pieterse, B.; Felzel, E.; Winter, R.; Van Der Burg, B.; Brouwer, A. PAH-CALUX, an optimized bioassay for AhR-mediated hazard identification of polycyclic aromatic hydrocarbons (PAHs) as individual compounds and in complex mixtures. *Environ. Sci. Technol.* **2013**, *47* (20), 11651−9.
(40) Billiard, S. M.; Timme-Laragy, A. R.; Wassenberg, D. M.; Cockman, C.; Di Giulio, R. T. The role of the aryl hydrocarbon receptor pathway in mediating synergistic developmental toxicity of polycyclic aromatic hydrocarbons to zebrafish. *Toxicol. Sci.* **2006**, *92* (2), 526−36.
(41) Hakkola, J.; Rysä, J.; Hukkanen, J. Regulation of hepatic energy metabolism by the nuclear receptor PXR. *Biochim. Biophys. Acta, Gene Regul. Mech.* **2016**, *1859* (9), 1072−82.
(42) Gore, A. C.; Chappell, V. A.; Fenton, S. E.; Flaws, J. A.; Nadal, A.; Prins, G. S.; Toppari, J.; Zoeller, R. T. Executive summary to EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. *Endocr. Rev.* **2015**, *36* (6), 593.
(43) Kersten, S. Integrated physiology and systems biology of PPARα. *Mol. Metab.* **2014**, *3* (4), 354−71.
(44) Laganà, A. S.; Vitale, S. G.; Nigro, A.; Sofo, V.; Salmeri, F. M.; Rossetti, P.; Rapisarda, A. M.; La Vignera, S.; Condorelli, R. A.; Rizzo, G.; Buscema, M. Pleiotropic actions of Peroxisome Proliferator-Activated Receptors (PPARs) in dysregulated metabolic homeostasis, inflammation and cancer: current evidence and future perspectives. *Int. J. Mol. Sci.* **2016**, *17* (7), 999.
(45) Bekins, B. A.; Cozzarelli, I. M. Nonvolatile dissolved organic carbon and diesel range organics concentrations measured in 2016 at the USGS crude oil study site near Bemidji, Minnesota, USA. *U. S. Geological Survey Data Release*. 2017, https://doi.org/10.5066/F7CN733T.
(46) Bekins, B. A.; Martinović-Weigelt, D.; McGuire, J. T.; Cozzarelli, I. M. Toxicity data for groundwater contaminated by petroleum hydrocarbons near Bemidji, MN (2016) *U. S. Geological Survey Data Release*; 2018, https://doi.org/10.5066/P9TU6W80.

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Environ. Sci. Technol. 2018, 52, 12172−12178