Unlike PAHs from Exxon Valdez Crude Oil, PAHs from Gulf of Alaska Coals are not Readily Bioavailable

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In the wake of the 1989 Exxon Valdez oil spill, spatially and temporally spill-correlated biological effects consistent with polycyclic aromatic hydrocarbon (PAH) exposure were observed. Some works have proposed that confounding sources from local source rocks, prominently coals, are the provenance of the PAHs. Representative coal deposits along the southeast Alaskan coast (Kulthieth Formation) were sampled and fully characterized chemically and geologically. The coals have variable but high total organic carbon content, technically classifying as coals and coaly shale, and highly varying PAH contents. Even for coals with high PAH content (4000 ppm total PAHs), a PAH-sensitive bacterial biosensor demonstrates nondetectable bioavailability as quantified, based on naphthalene as a test calibrant. These results are consistent with studies indicating that materials such as coals strongly diminish the bioavailability of hydrophobic organic compounds and support previous work suggesting that hydrocarbons associated with the regional background in northern Gulf of Alaska marine sediments are not appreciably bioavailable.

Introduction

The T/V Exxon Valdez discharged at least 41 000 m³ of Alaska North Slope crude oil onto the waters of Prince William Sound, Alaska, polluting 200+ km of shorelines along the coast up to 750 km to the southwest. Long-term biological damage attributed to lingering oil is inferred from spill-related spatial and temporal patterns in biochemical responses, characteristic of polycyclic aromatic hydrocarbon (PAH) exposure of monitored species, including cytochrome P450 1A (CYP1A) induction and detection of fluorescent aromatic compounds (FAC) in bile (1–3). However, geological back-ground sources of PAHs in marine sediments of the northern Gulf of Alaska have been impugned as confounding PAH sources. These deposits, including organic-rich hydrocarbon source rocks, coals, and natural oil seeps (4–7), have been proposed as alternative sources for the observed biochemical responses (8, 9). The basis for such conclusions is debatable (10, 11), and claims are difficult to evaluate given source chemical complexity and the lack of a controlled setting for monitoring exposure (12). This study addresses the difficulty by focusing on an end-member of interest (coals) and by employing a controlled setting for exposure.

The proposed provenance of confounding sources is organic-rich hydrocarbon source rock exposed along the Alaskan coast southeast of Prince William Sound from Katalla to Yakutat. These include organic-rich shale from the Poul Creek Formation, coals from the Kulthieth Formation, and oil seeps from both (13). Regional glaciation produces PAH bearing particulates that are carried to the coast by rivers and streams, where larger particles are deposited with marine sediment. Smaller particulates remain suspended and are transported by the Alaska Coastal Current westward to Prince William Sound, settling to the seafloor in the more quiescent waters (6, 7). Sterane and triterpane biomarkers indicate that the geologic deposit-derived PAHs predominately originate from the Kulthieth Formation (13, 14). Whereas putative organic-rich hydrocarbon source rock in the Kulthieth Formation may lie inaccessible beneath glaciers, numerous coal outcrops are accessible. These oil-prone coals are the likely source of oil seeps in the Yakataga region (14). While these seeps are too small to plausibly contaminate a large area of marine sediments, association of finely divided coal particles with siliciclastic material could account for all or most of the marine sediment PAH burden attributed to regional hydrocarbon source rock (13).

Here we use engineered bacterial biosensors (bioreporters) to investigate the bioavailability of PAHs in Kulthieth Formation coals, an end-member contribution to Gulf of Alaska marine sediments. Pollutant bioavailability as a risk assessment parameter has been of interest for regulatory purposes as early as 1989 (15). Lack of a standard definition or standardized methods for quantification causes difficulty in implementing bioavailability for risk-based applications (16), with additional complication in reconciling the related term, bioaccessibility. The term bioavailability references only the amount of a substance available to an organism at some time, and bioaccessibility relates to the amount of a substance that is and could potentially become available, i.e., for toxic response (16). In a quantifiable or mathematical sense, bioavailability is an instantaneous quantity and bioaccessibility is bioavailability integrated over time. Thus terms such as “bioavailable fraction” allude to bioaccessibility (i.e., the total fraction or load available or potentially available). Most measurement methods, whether chemical or ecotoxicological, are performed over short periods of time compared to the time scale for slow desorption in environmental settings, hence represent an incomplete measurement of bioaccessibility. Nonetheless such methods are used and accepted, as we have previously discussed, ad hoc, and the ad hoc quantity correlates with theoretical (t = ∞) bioac-

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distinct quantity, sometimes confused with bioavailability and/or bioaccessibility, but not relevant to this study.

Measuring bioavailability with bioreporters combines the advantages of chemical and ecotoxicological approaches. Chemical techniques are static vis a vis living organisms’ dynamic interactions with chemically complex matrices (17); ecotoxicological methods are dynamic but only measure bioavailability indirectly via toxic effects (18). The bioreporter used here employs sensor and regulator proteins of a metabolic pathway (i.e., not an intracellular pathway for toxicity response) and exhibits increasing response with increasing analyte concentration, characteristic of chemical approaches, while reflecting the biological system dynamics, characteristic of ecotoxicological approaches (19–22). Further, we previously documented that for experiments with biocided sediments and model materials, i.e., removing biological dynamics, bioreporter measured bioavailability correlates strongly with bioavailability approximated chemically (Tenax extraction) (23). Results from this study demonstrate strong and dose dependent bioreporter response from Exxon Valdez crude oil (EVCO) exposure and no discernible response for Kuhlthieth Formation coals.

**Experimental Section**

**Field Area and Sample Suite.** Icy Bay (60° 01’ N, 141° 20’ W) is a coastal fjord 100 km northwest of Yakutat, Alaska, and 275 km east of Prince William Sound, Alaska. Four fjords radiate from inner Icy Bay, one of which is Taan Fjord (called Tyndall Arm as the upper Taan Fjord terminates at the retreating toe of the Tyndall glacier). Eocene Kuhlthieth Formation oil-prone bituminous coals occur along ~130 km of the coastal margin between Cape Yakataga and the Samovar Hills, including Icy Bay (14). Coal samples were taken along a vertical section from dipping beds exposed on the east side of upper Tyndall Arm and from riverine coal floats (Figure 1). Exposed coal seams range from 6 cm to 1.5 m thick. Coal samples with a large PAH content were chosen for this study; additional information is in the Supporting Information (SI). An EVCO sample was also used. The sample was collected by Dr. David Shaw (University of Alaska Fairbanks) directly from the cargo hold of the T/V Exxon Valdez after its stranding on Bligh Reef. Portions were subsequently stored in airtight containers in the dark at ~20 °C.

**Materials.** Dialysis tubing was from Spectrum (Spectra/ Por, MWCO: 6000–8000). Chemicals and media were obtained from Fisher, Fisher Biotech, and Sigma Aldrich.

**Bioreporter Studies.** *Bacterial Strain and Culture Conditions.* The bioreporter, *Burkholderia sartisoli* sp. strain RP037 (24), carries a plasmid (pJAMAS7) with a second copy of the PhnR regulatable promoter, \( P_{\text{phnS}} \). When PAHs bind to a repressor protein, it activates transcription of the reporter gene to produce a reporter mRNA, which undergoes translation to produce EGFP, a stable variant of green fluorescent protein GFP (vide infra). Naphthalene was used for calibration, since the organism is most sensitive to naphthalene, although it also reacts to phenanthrene and some other PAHs. To culture, a ~80 °C stored portion was thawed and grown overnight (~16 h) in Luria Broth (LB) at 26 °C, shaking at 150 rpm, to an optical density at 600 nm of 1 (OD600 = 1). The overnight culture was diluted 1:50 in LB and regrown under like conditions to OD600 = 1.0. All LB media contained 50 mg/L kanamycin for maintaining the reporter construct. Cells were centrifuged at 1800 rcf for 5 min, and the cell pellet was resuspended in minimal medium (MM, details in SI) to OD600 = 0.20. Fresh cultures prepared in this manner were used for bioassays.

**Preparation of Amended Kaolinite and Coal Samples for Biological Analysis.** Naphthalene-amended kaolinite samples were prepared by spiking 1 g of kaolinite with 20 µL methanolic solutions of naphthalene at various concentrations and thoroughly mixing. Kaolinite samples amended with various concentrations of EVCO were prepared likewise by adding a weight-range of small portions of the crude oil. Coal samples were prepared by grinding and sieving (100 µm metal sieve). For coal, varying PAH concentrations were obtained by using samples having different natural PAH contents.

**Activation and Response Measurement.** Samples (1 g) were each placed in a 30 cm long piece of dialysis tube. Loaded tubes were suspended in sterilized 125 mL Erlenmeyer flasks and 30 mL of bioreporter culture was added to each flask, submerging the sample containing portion of the tube in culture. Flasks were incubated at 26 °C and shaken at 150 rpm. Periodically (times reported below by experiment), 3.0 mL was removed from each flask and used to measure both the OD560 and the fluorescence emission spectrum (Varian Cary Eclipse, 480 nm excitation). EGFP fluorescence emission peak area was taken as bioreporter response. Additional information on cultures and cell viability is in the SI.

**Chemical and Geological Studies.** *Pyrolysis and TOC Analysis of Coals.* Samples for total organic carbon (TOC) analysis and pyrolysis analysis were ground and sieved to <250 µm. For TOC, samples were weighed (~0.1 g) and acid washed (HCl, minimum 2 h to remove carbonates), then filtered through a glass microfiber filter. The filter was heated in crucible in a LECO 600 carbon analyzer to 1100 °C to oxidize organic matter; TOC was determined according to CO2 evolved. Rock-Eval (Rock-Eval II with TOC, Vinci
Technologies) pyrolysis was performed to assess coal thermal maturity and quality as oil bearing source rock (Ref. 25). Samples (~0.1 g) were incrementally heated under a helium atmosphere; the heating regime was 300 °C for 3 min (producing peak S1, i.e., volatilization of free/unbound hydrocarbons) followed by heating at 25 °C/minute to 600 °C (producing peak S2 representing heavy hydrocarbons, > C40, and cracking of kerogen, i.e., the bound/generated fraction). S1 and S2 are detected by flame ionization detection, but during thermal cracking carbon dioxide (peak S3) is also liberated (at 300–390 °C) and trapped; after S2 measurement the instrument heats the trap, driving off free hydrocarbons in sample while S2 is generated hydrocarbons. $T_{\text{max}}$ is a maturity parameter, HI and OI reflect the type of kerogen, S1/TOC is normalized oil content, and PI reflects maturity. Under notes, n indicates normal program and its2p indicates low temperature S2 shoulder.

### Table 1. Results from TOC and Rock-Eval on Icy Bay Samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>TOC (%)</th>
<th>S1 mg/g</th>
<th>S2 mg/g</th>
<th>S3 mg/g</th>
<th>$T_{\text{max}}$ (°C)</th>
<th>calc $R_0$ (%)</th>
<th>HI</th>
<th>S2/S3</th>
<th>S1/TOC</th>
<th>PI</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC-A</td>
<td>36</td>
<td>7.3</td>
<td>121</td>
<td>2.1</td>
<td>456</td>
<td>1.05</td>
<td>339</td>
<td>6</td>
<td>58</td>
<td>21</td>
<td>0.06</td>
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<tr>
<td>PKC-B</td>
<td>82</td>
<td>10.1</td>
<td>300</td>
<td>3.6</td>
<td>447</td>
<td>0.89</td>
<td>365</td>
<td>4</td>
<td>84</td>
<td>12</td>
<td>0.03</td>
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<tr>
<td>PKC-C</td>
<td>76</td>
<td>22.2</td>
<td>252</td>
<td>2.9</td>
<td>449</td>
<td>0.92</td>
<td>332</td>
<td>4</td>
<td>87</td>
<td>29</td>
<td>0.08</td>
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<tr>
<td>PKC-D</td>
<td>50</td>
<td>8.9</td>
<td>146</td>
<td>2.5</td>
<td>453</td>
<td>0.99</td>
<td>294</td>
<td>5</td>
<td>59</td>
<td>18</td>
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<td>PKC-E</td>
<td>76</td>
<td>15.2</td>
<td>283</td>
<td>2.4</td>
<td>446</td>
<td>0.87</td>
<td>345</td>
<td>3</td>
<td>110</td>
<td>20</td>
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<tr>
<td>PKC-F</td>
<td>80</td>
<td>11.5</td>
<td>350</td>
<td>2.7</td>
<td>450</td>
<td>0.94</td>
<td>375</td>
<td>3</td>
<td>115</td>
<td>14</td>
<td>0.04</td>
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<tr>
<td>PKC-G</td>
<td>76</td>
<td>10.1</td>
<td>277</td>
<td>2.2</td>
<td>446</td>
<td>0.87</td>
<td>366</td>
<td>3</td>
<td>127</td>
<td>13</td>
<td>0.04</td>
</tr>
<tr>
<td>PKC-H</td>
<td>25</td>
<td>3.2</td>
<td>87.1</td>
<td>7.1</td>
<td>444</td>
<td>0.83</td>
<td>353</td>
<td>29</td>
<td>13</td>
<td>13</td>
<td>0.04</td>
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</tbody>
</table>

Measured quantities are thermally released bitumen (S1, pyrosylate or S2, i.e., from thermal cracking of kerogen, carbon dioxide or S3, and temperature at maximum pyrosylate yield, $T_{\text{max}}$; calculated quantities are $R_0$, hydrogen index (HI or 100 × S2/TOC), oxygen index (OI or 100 × S3/TOC), S2/S3, S1/TOC, and production index (PI or S1/(S1 + S2)). S1 reflects free hydrocarbons in sample while S2 is generated hydrocarbons. $T_{\text{max}}$ is a maturity parameter, HI and OI reflect the type of kerogen, S1/TOC is normalized oil content, and PI reflects maturity. Under notes, n indicates normal program and Its2p indicates low temperature S2 shoulder.

### Table 2. Results from PAH Analysis of Crude Oil and Coal Samples Used in This Study

<table>
<thead>
<tr>
<th>Sample ID:</th>
<th>Exxon Valdez crude oil</th>
<th>Tyndall Glacier coal seam</th>
<th>PKC-A</th>
<th>Tyndall Glacier coal seam</th>
<th>PKC-B</th>
<th>Tyndall Glacier coal seam</th>
<th>PKC-C</th>
<th>Tyndall Glacier coal seam</th>
<th>PKC-D</th>
<th>Tyndall Glacier coal seam</th>
<th>PKC-E</th>
<th>Tyndall Glacier coal float</th>
<th>PKC-F</th>
<th>Tyndall Glacier coal float</th>
<th>PKC-G</th>
<th>Caetani River coal float</th>
<th>PKC-H</th>
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</thead>
<tbody>
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<td>Concentrations (mg/g):</td>
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<tr>
<td>Nap</td>
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<td>0.010</td>
<td>0.004</td>
<td>0.13</td>
<td>0.020</td>
<td>0.063</td>
<td>0.004</td>
<td>0.001</td>
<td>0.033</td>
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<tr>
<td>2-methyl nap</td>
<td>1.33</td>
<td>0.046</td>
<td>0.020</td>
<td>0.39</td>
<td>0.070</td>
<td>0.17</td>
<td>0.010</td>
<td>0.006</td>
<td>0.096</td>
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<tr>
<td>1-methyl nap</td>
<td>1.02</td>
<td>0.053</td>
<td>0.040</td>
<td>0.37</td>
<td>0.076</td>
<td>0.16</td>
<td>0.035</td>
<td>0.022</td>
<td>0.078</td>
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<tr>
<td>nap-2</td>
<td>3.15</td>
<td>0.23</td>
<td>0.16</td>
<td>0.61</td>
<td>0.24</td>
<td>0.43</td>
<td>0.095</td>
<td>0.18</td>
<td>0.17</td>
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<tr>
<td>nap-3</td>
<td>2.35</td>
<td>0.33</td>
<td>0.37</td>
<td>0.47</td>
<td>0.22</td>
<td>0.34</td>
<td>0.16</td>
<td>0.21</td>
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<td>nap-4</td>
<td>0.60</td>
<td>0.16</td>
<td>0.20</td>
<td>0.16</td>
<td>0.067</td>
<td>0.11</td>
<td>0.10</td>
<td>0.17</td>
<td>0.066</td>
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<td>Phe</td>
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<td>0.080</td>
<td>0.061</td>
<td>0.090</td>
<td>0.051</td>
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<td>phe-1</td>
<td>0.75</td>
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<td>0.20</td>
<td>0.23</td>
<td>0.12</td>
<td>0.18</td>
<td>0.15</td>
<td>0.19</td>
<td>0.081</td>
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<tr>
<td>phe-2</td>
<td>0.89</td>
<td>0.21</td>
<td>0.24</td>
<td>0.22</td>
<td>0.11</td>
<td>0.15</td>
<td>0.15</td>
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<tr>
<td>phe-3</td>
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<td>0.11</td>
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<tr>
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<tr>
<td>2PAH</td>
<td>14.7</td>
<td>2.18</td>
<td>2.14</td>
<td>3.56</td>
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</table>

$^a$ See the Supporting Information.
Heptane was added, then samples were kept tightly sealed on a mechanical shaker at 26 °C and 150 rpm for 15 h. Following this extraction, the heptane layers were analyzed by GC-MS (Varian CP-3800 gas chromatograph/Saturn 2200 mass spectrometer/CTC Analytics Combi-PAL autosampler). Naphthalene sorbed in such tests was expressed as a percent of the naphthalene control solution, and used to estimate the concentration of naphthalene in the solid \( (C_s) \) and aqueous \( (C_{aq}) \) phases (equilibrium calculations indicate that the vapor phase is negligible). Sorption is expressed as a single-point apparent distribution coefficient \( K'_{d} \), where \( K'_{d} = C_s / C_{aq} \). The experimental conditions were chosen after rough testing of the saturation behavior to ensure that the final sorption tests were performed in the linear part of the isotherm.

Results and Discussion

Figure 2 provides background information about RP037; Figure 2A illustrates how the RP037 sensing element expresses EGFP in response to PAH activation (previously reported (24)). Figure 2B shows the dialysis tube setup, and Figure 2C and D show RP037 logarithmic response as a function of naphthalene concentration in solution (no solid). Logarithmic response has been observed for similar bacterial systems, depending upon concentration range and other factors (refs 19–22, 28, 29, and references therein). The sensitivity of response is influenced by, for example, stage of growth phase upon activation; however, Figure 2D is representative and readily reproducible with high precision (typically \( R^2 > 0.99 \) as shown).

We have previously demonstrated that kaolinite is a good model solid for use in bioreporter studies, representing, as we prepare it, a solid from which naphthalene is 100% bioavailable (23). Thus, kaolinite amended with naphthalene or EVCO represents a benchmark by which 100% bioavailability is assessed and is not intended to represent sediments per se. Though we have used sediments to validate this approach in a previous study, the point here is not to study sediments (i.e., as previous studies with reference to bioavailability and environmental materials in the Gulf of Alaska or Prince William Sound), but rather specifically to contrast bioavailability of PAHs from EVCO to those from Kulthieth Formation coals, to wit, potential sources. Kaolinite provides a proxy to the solid support of a geomaterial (PAH-bearing sediments or coals) without introduction of collateral bioavailability modifying parameters such as the complex assemblages of organic matter/coal in natural sediments. EVCO-amended kaolinite can be viewed as the crude oil end-member analog to be contrasted with the coal end-member.

Solids can interfere with bioreporter response measurements (e.g., via sample autofluorescence), and bacteria allowed to freely mix with a solid sample are difficult to recover for response measurement. For these reasons, we use the dialysis tube assay. Amending kaolinite with naphthalene or crude oil results in a solid-phase material that may be handled in the same way as coal samples. Results of calibration experiments with naphthalene-amended kaolinite confirm the practicability of our approach. Figure 3A shows response taken after 16 h activation. We consistently observe an optimum response between \( \sim 12 \) and 18 h in terms of both sensitivity and precision. Curves from a separate calibration experiment in Figure 3B show that even after
30 h activation precision is still acceptable, but after 50 h there is a decline in sensitivity and precision, possibly due to poststationary lysis.

Figure 4 shows representative results from RP037 studies on EVCO-amended kaolinite (Figure 4A) and coal samples from Icy Bay, Alaska (Figure 4B), along with naphthalene-amended kaolinite for reference. For EVCO composition is fixed, so dose dependency is demonstrated by amending kaolinite samples with increasing amounts of EVCO. At lower concentrations, the magnitude of the EVCO response is slightly higher than for the naphthalene-amended kaolinite reference samples. At higher concentrations, the EVCO response becomes nonlinear. These observations are consistent with a priori expectations: EVCO response should be higher than for the calibrant because it is a complex material with bioreporter-inducing PAHs other than naphthalene, and at higher PAH concentrations, i.e., as the ratio of EVCO increases relative to a fixed amount of kaolinite, the thickness of the oil rim around kaolinite grains increases, and physical resistance to mass transport begins to increase nonlinearly (surface area:volume effect). In the case of coal samples, PAH composition varies substantially (Tables 1–2 and SI Tables S1–S2), and so a range of naphthalene/PAH concentrations was achieved by use of multiple samples, none of which was artificially amended. Figure 4B shows that there was essentially baseline bioreporter response to coal samples. Results from chemical and Rock-Eval analyses in Tables 1 and 2 indicate a substantial organic content of EVCO and coal samples. For coals, the TOC results correlate with S1 + S2 from the Rock-Eval analysis at $R^2 = 0.98$. Based on TOC results, five Icy Bay samples with TOC $>50\%$ classify as coal (TOC from 76 to 82%), two samples classify as coaly shale (TOCs of 25 and 46%), and one is in between (50%). Normalizing the S2 and S3 peaks to TOC content yields hydrogen index (HI) and oxygen index (OI), which indicate that generated hydrocarbons are likely to be oil or gas. The uniformly high HI and low OI values indicate the coals are oil prone, converting 25–30% by weight into oil upon heating; the remainder/coaly shales are also oil prone and convert 9–15% by weight to oil (Table 1, S2).

High HI values and low S1/TOC ratios suggest that most of the organic matter in the Icy Bay samples is not labile, and over most of the naphthalene concentration range in Figure 4 biosensor response for kaolinite amended with different amounts of crude oil is nearly superimposable with the results for naphthalene-amended kaolinite, whereas coal samples show baseline response. Qualitatively it is clear that PAHs from EVCO are highly available and PAHs from coal, even these oil prone coals, are entirely unavailable. Quantitatively, Exxon Valdez crude oil has essentially 100% bioavailability of PAH as compared to 0% bioavailability of PAH in Icy Bay coals. Viability measurements demonstrated no evidence of compromised cells (i.e., no toxicological effect) during the course of studies on EVCO and coal (see SI).

After the Exxon Valdez oil spill, a comprehensive effort was undertaken to study exposure to and effect of hydrocarbons in the affected area, of which PAHs are a toxic fraction (more especially the higher molecular weight PAHs, which are both more slowly degraded and weathered). Subsequently, some investigators have proposed that the source of toxicologically active PAHs is a geological/indigenous "regional background", based on inconclusive biochemical evidence from resident biota. The results here and recent reports on the effects of black carbon on bioavailability contradict this suggestion. Coal particles in marine sediments of the northern Gulf of Alaska could even act as a PAH sink and reduce PAH bioavailability (30–36). While Figure 4B demonstrates that there is no bioreporter response for these coals, a slight negative slope (not statistically differentiable from zero slope, $p<0.05$) with increasing concentration could indicate that more oil-prone coals have less available PAHs. From the results for coal in Figure 4B, we estimated the relative sorptive capacities of the coal samples and results for naphthalene are tabulated in Table 3 as apparent distribution coefficients, $K_d'$.
finding (Table 1 and SI Table S1), as do results from chemical sorption tests (Table 3, and consistent with environmental literature on coals and black carbon). A self-consistent conclusion emerges that although the coal particles found in continental shelf sediments of the Gulf of Alaska contain suites of PAHs resembling crude oils (e.g., ref 7), they have the capacity to remove PAHs from seawater and are more likely a sink than a source. Though there is a growing consensus in the environmental literature that PAHs from coal are not bioavailable, there is hardly a consensus within the greater scientific community. In the emerging field of medical geology reports document release of PAHs from coal, in some cases linking release of organics from coal with putative adverse health effects. Such studies underscore the importance of linking biological effect to coal characterization (chemical) and understanding of geological context (36–43).

Due to the high chemical resistance to mass transport (release) of PAHs from the coal samples used here, the corresponding dominant desorption kinetics will be very slow, obviously much slower than the time scale of measurement employed here. We and other authors have addressed this point from many perspectives, full consideration of which is beyond the scope of the present manuscript. In brief, it is difficult to extend bioreporter studies past the time periods used here. However, most measurements of bioavailability are similarly restricted to measurement periods of a few hours or even weeks, i.e., periods equally inadequate to slow desorption (many months to years; with reference to the Valdez spill, some studies address this point of very slow desorption (13), others overlook it). One highly cited work on chemical extraction demonstrated that the bioremediable (i.e., perfuce ad hoc bioaccessible) fraction of desorbing PAHs over two years is captured in the amount desorbed to Tenax over the same time frame as used in the present experiments (44). This result stems from the quasi-infinite sink nature of the Tenax extractant’s driving release in conjunction with the aforementioned asymptotic approach of ad hoc bioaccessibility to the theoretical limit. Bacteria that metabolize PAHs, bioreporters inclusive, also function as a quasi-infinite sink, as we have before documented (17), which is in accord with our previous demonstration of a 1:1 correspondence between bioreporter measured bioavailability and Tenax measured bioavailability (23).

PAH-sensitive bioreporter bacteria demonstrate a very strong and dose dependent bioavailable response from EVCO exposure and no response for Kultthieth Formation coals for the same range of PAH loading. These results corroborate earlier work suggesting that hydrocarbons associated with the regional background in northern Gulf of Alaska marine sediments are not appreciably bioavailable. Taken in the context of literature reports on black carbon, the present results are consistent with other spill-related spatial and temporal patterns in long-term indications of toxic stress resulting from exposure to the release of EVCO into the environment. One reviewer of this manuscript mandated mention of another possibility for the role of these coals in the environment: because of their capacity to sorb PAHs, rates of other processes of natural attenuation (photooxidation, biodegradation) could be diminished, diminishing the immediate PAH burden, but enhancing the persistence of toxic PAHs. While we mention this possibility, it is tangential to our main study objectives and findings. Our primary finding, based on quantitative results from the present end-member study of bioavailability, is that bioavailable PAHs do not originate from organic-rich source rock associated with the Poul Creek and Kultthieth Formations east of Prince William Sound. EVCO represents the primary known source of bioavailable PAHs in the region.

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Supporting Information Available
Additional information on methods, chemical and geological characterization of samples, and studies on cell viability. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited