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1 **Chemical and Toxicological Characterizations of Hydraulic Fracturing**

2 **Flowback and Produced Water**

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ABSTRACT

Hydraulic fracturing (HF) has emerged as a major method of unconventional oil and gas recovery. The toxicity of hydraulic fracturing flowback and produced water (HF-FPW) has not been previously reported and is complicated by the combined complexity of organic and inorganic constituents in HF fluids and deep formation water. In this study, we characterized the solids, salts, and organic signatures in an HF-FPW sample from the Duvernay Formation, Alberta, Canada. Untargeted HPLC-Orbitrap revealed numerous unknown dissolved polar organics. Among the most prominent peaks, a substituted tri-phenyl phosphate was identified which is likely an oxidation product of a common polymer antioxidant. Acute toxicity of zebrafish embryo was attributable to high salinity and organic contaminants in HF-FPW with LC50 values ranging from 0.6% to 3.9%, depending on the HF-FPW fractions and embryo developmental stages. Induction of ethoxyresorufin-O-deethylase (EROD) activity was detected, due in part to polycyclic aromatic hydrocarbons (PAHs), and suspended solids might have a synergistic effect on EROD induction. This study demonstrates that toxicological profiling of real HF-FPW sample presents great challenges for assessing the potential risks and impacts posed by HF-FPW spills.

Keywords: Hydraulic fracturing, Flowback water, Aryl phosphate esters, PAH, Aquatic toxicity, Synergism

1. Introduction

The combination of horizontal drilling and high-volume hydraulic fracturing (HF) has emerged recently as a major method of recovering oil and gas from tight reservoirs (Osborn et al., 2011). Despite its promise in resource recovery, there are environmental concerns surrounding the HF process (Goss, 2015; Alessi et al., 2016). Considerable attention has been given to contamination of shallow groundwater with natural gas from deep reservoirs following fracturing (Osborn et al., 2011; Jackson et al., 2013; Darrah et al., 2014; Llewellyn et al., 2015). A recent review documented the potential impacts to underground sources of drinking water and domestic wells as a result of the hydraulic fracturing process itself (DuGiulio and Jackson, 2016). However, risks posed by surface release of hydraulic fracturing flowback and produced waters (HF-FPWs), which result from the interaction of fracturing fluids with the target formation and that return to the surface following fracturing, are less evaluated (Sang et al., 2014; Drollette et al., 2015). HF-FPWs are often briny and may contain heavy metals, radionuclides and numerous organic constituents (Colborn et al., 2011; Drollette et al., 2015; Llewellyn et al., 2015; DuGiulio and Jackson, 2016). Because of their complexity, it is challenging to accurately identify the chemical profiles and potential for adverse biological impacts posed by these fluids.

The highest risk of HF-FPW exposure to aquatic organisms is surface spills that may occur during transport to disposal or treatment sites. Contamination of surface water and shallow groundwater following spills of HF-FPW in certain regions is well documented with more than 2500 spills of HF-FPW being documented in the period 2011-2014 in Alberta alone (Goss, 2015; AER, 2016; Alessi et al., 2016) and this can provide a baseline for exposure assessment. However, to properly assess risk, having an *a priori* understanding of the biological effects of these fluids to aquatic organisms is necessary for both risk management and in helping to define

60 necessary remediation strategies following surface spills. To date, the identification of biological
61 impacts following a HF-FPW spill to aquatic animals, and ultimately to human health, have not
62 been assessed (Harkness et al., 2015).

63 Assessing the organic, inorganic and toxicological signatures of saline hydrocarbon
64 contaminated waters poses significant analytical challenges. Recent analogous successes include
65 assessing oil sand process water (Martin et al., 2008,2010; Rowland et al., 2011; He et al.,
66 2012a,b), and the complex environmental mixtures resulting from the Deep Horizon oil spill
67 (Allan et al., 2012; Incardona et al., 2014). Various organic contaminants including polycyclic
68 aromatic hydrocarbons (PAHs), naphthenic acids, and endocrine disruptive chemicals have been
69 detected in these samples (Martin et al., 2008; Kelly et al., 2009; Rowland et al., 2011; Allen et
70 al., 2012). For HF-FPW, additional complexity arises from the variable co-return of the original
71 additives in the fracturing fluid itself mixing with salts and/or saline interstitial fluids during the
72 fracturing of the target formation (AER, 2016; Alessi et al., 2016). A recent study reported on the
73 discharge or spilling of wastewater from unconventional shale gas and hydraulic fracturing,
74 which may contain high concentrations of bromide, iodide, ammonium and naturally occurring
75 radioactive materials derived from the deep formation (Vengosh et al., 2014). Characterization
76 of a flowback water sample from Colorado demonstrated a highly complex profile of salts,
77 metals, and dissolved organic matters (Lester et al., 2015). It was suggested HF fluids and
78 wastewater may have endocrine disruptive activity based on the systematic evaluation of
79 chemicals used in HF fluids (Elliott et al., 2016). While several reviews have documented the
80 biological impacts and potential health risk of the chemicals placed down-hole during the
81 fracturing process (Stringfellow et al., 2014; Kahrilas et al., 2015; AER, 2016), there is almost
82 no information regarding the complexity of the chemistry and toxicity of actual HF-FPW

83 samples including the identity of suspended solids, signature of the returning organics, the
84 naturally-occurring hydrocarbons present in HF-FPW, and the resulting products of down-hole
85 chemical reactions that may be occurring. Similarly, there is no information on the potential
86 toxicological impacts of HF-FPW spills on freshwater organisms.

87 Here we present our study characterizing the complex solids, inorganics, organics and
88 toxicity signatures of a real HF-FPW sample from Duvernay Formation, Alberta, Canada. After
89 characterizing the solid phase and the solution chemistry of the HF-FPW sample, its acute
90 toxicity was assessed using the zebrafish embryo exposure model. Exposure to both the raw HF-
91 FPW field sample, which contained suspended solids, as well as a solids-free HF-FPW sample,
92 were conducted to investigate the differential effects of suspended solids on the lethal and sub-
93 lethal toxicity to zebrafish embryo. The objective of this study is to demonstrate the complexity
94 of a real HF-FPW sample by characterizing its organic and inorganic chemistry and assessing in
95 parallel its potential for toxicological effects on aquatic organisms, allowing for eventual linking
96 of those effects to HF-FPW chemistry.

97

98 **2. Materials and methods**

99 **2.1. HF-FPW samples**

100 A day 7 post-stimulation HF-FPW sample was derived from the hydraulic fracturing of the
101 Upper Devonian-aged Duvernay Formation, a heterogeneous marine deposit comprised of
102 siliceous and argillaceous mudstones, and limestones (Dunn et al., 2012). The gas well was
103 drilled by the Encana Corporation in early 2014 near Fox Creek, AB, to a true vertical depth
104 (TVD) of 3,200 m and a measured depth (MD) of 5,650 m, yielding a >2 km horizontal section
105 that was fractured in 19 stages. Following fracturing, the well flowed back from late May to

early June 2014. The temperature of the FPW sample upon reaching the separator was approximately 60°C, and the well produces approximately 74% C1, 14% C2, 5% C3, and between 0.5-2.0% C4 – C7 hydrocarbons. Hydrogen sulfide in the produced gas is below detection limits (<0.01%). The high salinity of the FPW sample examined here (see below) is consistent with studies that show the Upper Devonian in the region of study commonly have total dissolved solids (TDS) exceeding 200 g/L (Anfort et al., 2001).

In the experiments conducted, HF-FPW-S or S (sediment) refers to the HF-FPW sample as received, containing the suspended particle load. To generate a sediment-free sample (HF-FPW-SF or SF), a 1 L sub-sample of HF-FPW-S was centrifuged and filtered through a 0.22 µm membrane. To generate an activated-charcoal treated sample (HF-FPW-AC or AC), another 1 L sub-sample of HF-FPW-S was mixed and stirred with activated charcoal (5g/L) overnight and filtered through a 0.22 µm membrane. All samples were stored in the dark at room temperature prior to use.

2.2. Inorganic analysis

The sample HF-FPW was analyzed for its dissolved inorganic constituents and to characterize its associated solids. The pH of the sample as received was measured using Mettler Toledo Easy Five dual pH meter.

The total dissolved solids (TDS) of the HF-FPW sample was directly determined by dehydrating a 100 ml aliquot of HF-FPW, which was previously passed through a 0.45 µm nylon membrane to remove suspended solids. Subsequently the filtrate was placed in an oven at 80° C until all visible liquid had evaporated (approximately 3 days). The sample was further dried to dehydrate the residual salts at 250° C for 8 hours and the final weight measured once the weight

had stabilized. Suspended solids (SS) and total solids (TS) were determined as the mass of solids captured through vacuum filtration on a 0.45 µm ~~acetonitrile~~-cellulose nitrate filter. The SS sample was prepared from a portion the HF-FPW sample which had been allowed to settle for a minimum of 5 days. The TS sample was prepared from a portion of the HF-FPW sample which had been vigorously agitated. Filters were rinsed thoroughly with 18MΩ ultrapure water and then allowed to air dry for 7 days. Ion Chromatography, total organic carbon, total nitrogen and Inductively Coupled Plasma-Double Mass Spectrometry (ICP-MS/MS) were also performed for elemental analyses. Details are provided in the SI section.

2.3. Scanning Electron Microscopy

To conduct scanning electron microscopy (SEM), HF-FPW sediments and solids were collected by passing the sample of HF-FPW through a 0.45 µm cellulose nitrate~~acetonitrile~~ filter. The sediment was then rinsed while on the filter with 18MΩ ultrapure water to remove any residual brine and to prevent salt precipitation within the sample, covered, and air dried. The samples were then mounted adhesive carbon tape on aluminum SEM tabs (Ted Pella, Inc., Redding, CA), which were then carbon coated using a Nanotek SEMprep 2 sputter coater. Imaging was performed using a Zeiss EVO LS15 SEM with a LaB6 crystal and equipped with a Bruker silicon drift detector for energy dispersive X-ray analysis (EDX) with a peak resolution of 125 eV and a resolution of 100 nm. SEM analyses were performed on the TS fraction of the HF-FPW sample.

2.4. Untargeted analysis by HPLC-Orbitrap-MS

Flowback sample (2 mL) was added to an Eppendorf tube and centrifuged to remove suspended solids. The liquid phase was transferred to a 2 mL glass vial for analysis. The HPLC separation was performed using an ARIA MX transcend system (Thermo Fisher Scientific, San Jose, CA, USA) on a Hypersil Gold column (50 x 2.1 mm, 1.9 μ m particle size; Thermo Fisher Scientific, San Jose, CA, USA) at 40°C. The flow rate was 0.5 mL/min and an injection volume of 20 μ L was used. The mobile phases consisted of (A) 20 mM ammonium acetate in water and (B) 100% methanol. The mobile phase composition was 5% B for 2 min, followed by a linear gradient ramp to 90% B at 22 min, to 100% B over 10 min, and returning to 5% B in 2 min, followed by a 4 min hold prior to the next injection. A diversion valve directed the first 7 min of eluant to waste to protect the mass spectrometer from salts.

The Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) was operated in electrospray ionization (ESI) mode at 350°C and 5 KV. The sheath, auxiliary, and sweep gas (nitrogen) flow were set to 40, 5 and 2 (arbitrary units), respectively, capillary temperature at 300 °C, and S-Lens RF at 65%. The mass spectral resolving power was set to a nominal value of 120,000 at full width half-maximum at m/z 400, and using a full maximum ion time of 200 ms. MS/MS experiments were performed by collision-induced dissociation (CID) with a normalized collision energy of 35 eV, and by higher-energy collision dissociation (HCD) with collision energy of 35 eV, 50 eV and 80 eV.

2.5. PAHs Analysis

FPW samples (500 mL) were analyzed for polycyclic aromatic compounds directly, and after centrifugation (2500 g for 15 min). Analytes included parent PAHs, dibenzothiophene (DBT), and various alkyl-PAHs. An internal standard mixture (10 ng of each) was spiked into each

174 sample, followed by sequential liquid-liquid extraction with dichloromethane (2 x 50 mL). The
175 dichloromethane extracts were combined (100 mL) and concentrated to 1 mL by nitrogen gas
176 evaporation. Precleaned copper powder and anhydrous sodium sulfate were vortexed with the
177 extracts, which were then taken up in 5 mL hexane and reconstituted to 1 mL. Silica solid phase
178 extraction cartridges (Waters, 1g/6cc) were used for cleanup. These were pre-conditioned with 5
179 mL hexane, extracts were loaded, washed with 4 mL hexane. PAHs were eluted with 5 mL of
180 hexane/dichloromethane 4:1 (v/v), concentrated to 0.2 mL, taken up in 3 mL hexane and
181 concentrated to 200 µL which was transferred to vials with glass inserts. These vials were
182 centrifuged and 50 µL of supernatant was transferred into new glass inserts for GC-MS analysis.
183 GC-MS analysis was by splitless injection (1 µL) and chromatographic separation was
184 performed on DB-5ms (Agilent; 20 m × 0.18 mm × 0.18µm) with a helium flow of 1.8 mL/min.
185 Targeted analytes were analyzed in single ion monitoring (SIM) mode, and concentrations were
186 determined by relative response to an internal standard.

187

188 **2.6. Acute embryo toxicity test**

189 Zebrafish embryos were obtained from breeding of matured fish, cultured under standardized
190 conditions in the aquatic facility in the Department of Biological Sciences, University of Alberta.
191 Exposure was conducted in static conditions, in 250 mL glass beakers containing 150 mL of
192 exposure solution at 25±1 °C under 16h/8h light/dark cycle. The dilution water was moderately
193 hard water (USEPA, 2005), and details of preparation are provided in SI. For the exposure using
194 1 hour post fertilization (hpf) embryos, 30 fertilized embryos were randomly selected and placed
195 into each exposure beaker. Nine dilutions of HF-FPW-S and HF-FPW-SF were tested,
196 respectively; including 0 (control), 0.04%, 0.08%, 0.16%, 0.31%, 0.63%, 1.25%, 2.5%, and 5%.

197 The exposure period was 1 to 96 hpf. For the exposure using 24 hpf embryos, 30 fertilized
198 embryos were randomly selected and placed into each exposure beaker. Seven dilutions of HF-
199 FPW-S and HF-FPW-SF were tested, respectively; including 0 (control), 0.63%, 1.25, 2.5%, 5%,
200 10%, and 15%. The exposure time was 24 to 96 hpf. Seven dilutions of HF-FPW-AC were also
201 tested using 24 hpf embryos exposed from 24 to 96 hpf as salt control. All HF-FPWs samples
202 were diluted with water prior to exposure. During the exposures, any dead embryos observed
203 were removed immediately. All the exposures were repeated for 6 replications (n=6). At the end
204 of exposure, the mortality rate of embryos and LC50 values were calculated using Toxicity
205 Relationship Analysis Program (TRAP v1.22).

206

207 **2.7. Embryonic EROD assay**

208 The exposure settings for EROD assay using zebrafish embryo were similar to those for acute
209 toxicity tests. Thirty fertilized embryos were exposed from 24 to 96 hpf. The standard curve of
210 EROD activity was obtained by exposure to eight concentrations of Benzo(a)Pyrene (BaP),
211 including 0 (control), 156, 313, 625, 1250, 2500, 5000, and 10,000 ng/L. HF-FPW-AC, HF-
212 FPW-SF, and HF-FPW-S were tested in 1.25% and 2.5% dilutions. A co-exposure experiment
213 was designed to investigate the potential synergistic or additive effects of HF-FPWs on EROD
214 induction by exposure of 0 (control), 10, 100, 250, 500, and 1,000 ng/L of BaP with and without
215 1.25% of HF-FPW-AC, HF-FPW-SF, and HF-FPW-S. Embryonic EROD activity was measured
216 at the end of exposure. At the end of exposure, hatched larvae were gently rinsed with dilution
217 water and then placed into 24-well plate for 7-ethoxyresorufin (7-ER) treatment. A total number
218 of 20 live larvae were selected from each exposure beaker and randomly placed into 2 wells in
219 the 24-well plate. Each well contained 10 larvae and 2 mL of 7-ER solutions. 7-ER powder was

220 first diluted in absolute ethanol as stock, and further diluted with dilution water (See SI) prior to
221 use. The final concentration of 7-ER treatment solution was 2.5 $\mu\text{mol/L}$ in the 24-well plate.
222 After 8 hour of 7-ER treatment, 200 μL of 7-ER treatment solution was transferred into each well
223 of a 96-well microplate for EROD measurement using a VICTOR3V 1420 Multilabel Counter
224 (PerkinElmer, MS, USA). Resorufin was detected at 595 nm with a 535 nm excitation
225 wavelength. Four measurements (4 wells of 96-well plate, 200 μL of medium in each well) were
226 taken and averaged as readings for a single treatment of 7-ER. Two treatments of 7-ER (2 wells
227 of 24 well-plate, 10 larvae in each well) were taken and averaged as readings for a single
228 exposure. All the exposures were repeated 4 times ($n=6$).

229

230 **2.8. Statistical analyses**

231 The results of EROD activity induction in zebrafish embryos were expressed as fold change
232 relative to the control group. The relative equivalency of EROD activities were calculated based
233 on the equation generated by BaP standard curves. Statistical analyses were conducted using
234 SPSS16.0 (SPSS, Chicago, IL). All data are expressed as mean $\pm$ standard error of mean (SEM).
235 Normality of each data set was assessed by use of the Kolomogrov–Smirnov one-sample test,
236 and homogeneity of variance was determined by use of Levene’s test. Statistical differences
237 were evaluated by one-way ANOVA followed by Tukey test ($p<0.05$).

238

239 **3. Results and discussions**

240 **3.1. Inorganic analyses**

241 The results of physiochemical and elemental analyses are presented in Table 1. Briefly, the 7-day
242 HF-FPW sample was weakly acidic (pH 4.78), due to the application of hydrogen chloride in

original fracturing fluid (Frac Focus, 2016) and this demonstrates that the sample was a mixture of applied HF fluid and formation water. The sample also has a total dissolved solids (TDS) load of 242,624.3 mg/L ($\pm 0.5\%$ over three replicates), (242 ‰, approximately 7X seawater). The total organic carbon (TOC) and total nitrogen (TN) of the HF-FPW sample were measured to be 0.087% and 0.21% of the total TDS, respectively. The TOC and TN measurements only quantified the dissolved and miscible organic carbon compounds, additionally organics maybe associated with solid phases or immiscible compounds. TOC measurements may reflect true the total TOC associated with the sample as a thinly film was observed on the surface of the bulk HF-FPW sample as delivered from the field. It is likely that the sampling probe was below the surface of this film and therefore missed an immiscible fraction of the TOC associated with the sample and could explain why N was more abundant in the measured concentrations. Ion chromatography (IC) was used to determine chloride (Cl^-), bromide (Br^-), and sulfate (SO_4^{2-}) concentrations, and ICP-MS/MS was used to determine the concentration of 14 elements. The most abundant element was Cl^- (56%, of the total TDS), followed by Na (28.8%), Ca (4.85%), and K (1.05%). ICP-MS/MS measurements were made using a standard addition method for each element, to account for the impacts of high salinity and resulting isobaric interferences unique to HF-FPW samples; the resulting solution charge balance is within < 1% (Table S2). Although there is certain amount of radionuclides and heavy metals detected in our sample, with the dilution applied in the toxicological study, the concentrations and predicted toxicity will be very limited compared to other HF samples (Lauer et al., 2016), therefore, the potential toxicity of radioactive compounds and heavy metals will not be discussed in this manuscript.

3.2. Solids characterization

Suspended solids were present in the HF-FPW sample at a concentration of ~~22.549.1~~ mg/L ($\pm 5.0\%$ over three replicates). Scanning Electron Microscopy with Energy dispersive X-ray spectroscopy (SEM-EDX) was used to characterize the morphological features and size parameters of particles in the HF-FPW sample. The most common of the identifiable particles were amorphous precipitates, and spheres or sphere fragments coated with morphologically similar precipitates and that were approximately 2-10 μm in diameter (Fig. 1). EDX was performed on several spots on individual spheres to measure their approximate elemental composition. The sphere exterior is composed primarily of carbon (64%, ± 4.8), oxygen (33%, ± 1.9) iron (2%, ± 0.006), silica (1%, ± 0.001), and calcium (<1%) (Fig. 1A). Examination of broken spheres showed them to be hollow and composed of a thin shell; EDX analysis of the interior of the sphere shows it is composed of mainly carbon (79.5%, ± 7.4) and oxygen (19%, ± 0.8) with iron and silica making up less than a percent (± 0.003) (Fig. 1B). The strong carbon signal is likely dominated by the carbon used to coat the samples prior to analysis. The remaining elements (Fe, O and Si) are thought to makeup secondary precipitate composed of amorphous silica-doped iron oxide particles, which are found in clusters and as coatings on the hollow sphere structures. The decline in the Fe and Si signal on the interior verse the exterior of the spheres we interpret as indicating the silica doped iron oxides are a secondary that formed a coating on the exterior of spheres. The hollow spheres are themselves are a relic of a hydraulic fracturing fluid and were used as a delayed release delivery mechanism for an ammonium persulfate, which is used as a breaker. SEM-EDX analysis on additional types of particles is provided in SI. Iron oxides and silicates are known to contain amphoteric surface functional groups, which can have electrostatic interactions with cationic and anionic compounds (Zachara and Smith, 1994). This implies that both metal cations and anions, and polar organic compounds

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could adsorb to the surface of both the amorphous iron oxides and silicates depending on the solution chemistry. Iron oxides are long-known to be able to remove significant concentrations of metals from aqueous solution (Zhang et al., 2008) and have been proposed as a means of removing metals from contaminated waters (Mayes et al., 2009). In addition, it has been reported PAHs can be bound and accumulate in colloids and suspended solids (Kalmykova et al., 2013). Micron to nanoscale iron particles have been demonstrated to have a particularly high affinity for high molecular weight (HMW) PAHs, with up to 45% of HMW PAHs being bound in several road runoff samples (Nielsen et al., 2015). In the current study, approximately 50% of total PAHs and alkylated PAHs present in the HF-FPW sample were associated with the suspended load. This finding suggests that our observation of significant organic compounds including PAHs bound to the iron oxide particles and coatings in the HF-FPW sediment is not entirely unexpected. Further work is needed to quantify the loading of PAHs on HF-FPW sediment and their potential to desorb and remobilize.

302

3.3. Untargeted trace organics analysis

Fig. 2 demonstrates the range of polar organic substances that were observed in the current sample by the untargeted HPLC-Orbitrap MS method. A chromatogram is presented in three-dimensions to illustrate the most prominent analyte peaks. Among these, a series of polyethylene glycol (PEGs, $H-(OC_2H_4)_n-OH$) surfactants were detected (Fig. 2A, shaded region), consistent with the chemicals previously reported by Thurman et al. (2016) in HF-FPW samples from the United States. The PEG surfactants are polymers of ethylene oxide (EO) units, and those identified here included PEG-EO4 to PEG-EO13 (i.e. $n = 4-13$), also similar to the results of Thurman et al. (2016). Here all the PEG-EO_n species were confirmed by accurate masses of the

protonated or ammonium adducted ions, as well as by diagnostic fragmentation in MS/MS experiments.

However, PEGs were not the only important features in the chromatogram of the current sample. The overall complexity of the current HF-FPW sample is evident from Kendrick plotting (Fig. 2B). Each point in the Kendrick plot corresponds to a distinct chemical species (Fig. S2), whose molecular formula (but not structure) is known from exact mass (~2 ppm by the current method) and isotopic signatures.

The dominant chromatographic peak eluting at 35.24 min, shown in an extracted ion chromatogram as compound #3 (Fig. 2C), was chosen for further characterization. The focus on this chemical was, in part, because analytes eluting late by the current method are relatively hydrophobic and may accumulate in aquatic organisms. First, the empirical formula for this peak was assigned as $C_{42}H_{63}PO_4$ based on accurate mass ($[M+H]^+$ at m/z 663.4545, with mass accuracy of 0.2 ppm) and an exact mass molecular ion isotopic pattern matching the theoretical pattern of $100([M+H]^+):45([M+H+1]^+):10([M+H+2]^+)$. Second, the current HPLC-Orbitrap method allows MS/MS experiments on isolated ions, which can assist in structural elucidation. Here, the MS/MS spectrum of compound #3 was furthermore diagnostic, showing consecutive loss of C_4H_8 at low collision energy (CID and HCD modes) and further loss of phenyl groups from a phosphate core at higher collision energy (HCD mode) (Fig. S3). This allowed a general structure to be confidently proposed as an alkyl substituted tri-dibutylphenyl phosphate ester. This was later confirmed with an authentic standard as tris(2,4-di-tert-butylphenyl) phosphate (structure shown in Fig. 2). Alkyl phosphate esters were previously reported in HF-FPW samples (USEPA, 2015), but no aryl phosphate esters have been previously reported to our knowledge.

335 This phosphate chemical is not listed on *Canada's Domestic Substance List* (DSL),
336 however a corresponding tris(2,4-di-tert-butylphenyl) phosphite ($C_{42}H_{63}PO_3$) is a common
337 polymer antioxidant on the DSL and would yield the detected phosphate upon oxidation. In a
338 recent report, single-use bioprocess containers impregnated with this phosphite were shown to
339 leach a cytotoxic phosphate degradation product (bis(2,4-di-tert-butylphenyl) phosphate) upon
340 gamma irradiation (Hammond et al., 2015). This same toxic product was also confirmed here in
341 the HF-FPW sample (Fig. 2C, compound #2) at 25.12 min ($m/z = 475.2971, [M+H]^+$) by MS/MS
342 and with an authentic standard.

343 A related organic phosphate (Fig. 2C, compound #1) was also identified at a retention
344 time of 21.30 min. This was initially identified as $C_{18}H_{15}PO_4$ by accurate mass ($[M+H]^+$ at m/z
345 327.0781 with <3 ppm error) and fragmentations in MS/MS experiments showed consecutive
346 losses of phenyl moieties from a phosphate core. It was later confirmed with authentic standard
347 as triphenyl phosphate (Fig. 2C).

348 Triphenyl phosphate is a known flame-retardant used as a polymer additive, but also is
349 used in fire-resistant hydraulic fluids and in lubricants. Most aryl phosphates are readily
350 biodegradable and will partition to organic sediments and solids, but they also have moderate
351 bioconcentration potentials in fish (UKEA, 2009). Isales et al. (2015) recently described the
352 aquatic toxicity of triphenyl phosphate (compound #1) which is capable of developmental
353 toxicity through the retinoic acid receptor, producing effects in zebrafish that are similar to
354 dioxin-like cardiac effects. The reason for the presence of triphenyl phosphate in the current HF-
355 FPW is unclear. It may be an impurity in formulations of compound #3.

356

357 3.4. PAHs analyses

PAHs and other natural hydrocarbons have previously been characterized in formation water and flowback water by two-dimensional GC-MS (Orem et al., 2014). We also found evidence for parent PAHs and various alkyl PAHs in the current sample, with a significant fraction (approximately 50%) adsorbed to the particle fraction (Fig. 3 and Table S3). The total PAHs and alkylated PAHs in HF-FPW-SF were 520 and 1159 ng/L, while there were approximately double amounts of total PAHs and alkylated PAHs in HF-FPW-S (977 and 2058 ng/L).

3.5. Acute toxicity test

HF-FPW displayed acute toxicity to zebrafish embryo development. Fig. 4A illustrates the acute toxicity data of HF-FPWs derived from this study. For embryos exposed to HF-FPWs from 24 to 96 hours post fertilization (hpf), the LC50 values for HF-FPW-activated charcoal treated subsample (HF-FPW-AC), HF-FPW-sediment free subsample (HF-FPW-SF) and HF-FPW raw sample (HF-FPW-S) were similar. However, for the embryos exposed to HF-FPWs from 1 to 96 hpf, the LC50 values for HF-FPW-S was significantly lower than that of HF-FPW-SF, and both LC50 values of HF-FPW-S and HF-FPW-SF were significantly lower than that of HF-FPW-AC, indicating a stronger lethal effect on zebrafish embryos as result of organic fraction exposure in the first 24 h.

These results demonstrate that exposure to HF-FPW starting from 1 hpf had a greater lethal effect on zebrafish embryos in both HF-FPW-SF and HF-FPW-S compared to those exposed from 24 hpf onwards, suggesting that the early stage of embryo development is most vulnerable to HF-FPW exposure, probably due to the combined effects posed by salinity and organics from HF-FPW. For the exposure group from 1 to 96 hpf, exposure to HF-FPW-S, which also contained suspended solids, resulted in significantly higher lethality compared to

381 those exposures which were sediment free (HF-FPW-SF). This toxicity difference may be due to
382 concentrations of pollutants being markedly higher in HF-FPW-S than in HF-FPW-SF with total
383 PAHs and alkylated PAHs in HF-FPW-S being 2058 ng/L compared to 1159 ng/L in HF-FPW-
384 SF. A second possible explanation is the adsorption and aggregation of suspended particles onto
385 embryo surfaces. A large fraction of measured contaminant load in HF-FPW (e.g., PAHs) is
386 adsorbed by the suspended solids. It is possible these solids may then be acting as delivery
387 vehicle for contaminants to the surface of the embryo. Given our demonstration that these
388 particles tend to aggregate and adhere to the surface of chorion (Fig. 4D), this likely enhances
389 exposure resulting in higher noted mortality.

390 Embryos exposed to HF-FPW from 24 to 96 hpf demonstrated significantly higher LC50
391 values compared to those from 1 to 96 hpf (Fig. 4A), demonstrating the acute lethal toxic effects
392 of HF-FPWs were less if they were not exposed to the embryo during the very early stages of
393 development. In addition, based on the LC50 values for the embryos exposure from 24 to 96
394 hpf, exposure to HF-FPW-S also caused a high acute lethal toxicity compared to HF-FPW-SF.
395 We used activated charcoal treated (HF-FPW-AC) as a salt loading control since this treatment
396 has been demonstrated to remove most of the organic contaminants and heavy metals, leaving
397 behind the bulk salinity which is primarily sodium and chloride (Table 1, Table S1, S2) (He et al.,
398 2012). Since the results in the 24-96 hpf exposures (HF-FPW-S, HF-FPW-SF) were not
399 statistically different from the HF-FPW-AC, this suggests that salinity is the pre-dominant
400 toxicological driver 24 hpf. The complexity of HF-FPW, which contains high salinity, is likely
401 masking other toxicological effects and makes the determination of potential hazards posed by
402 HF-FPW challenging.

403

3.6. Embryonic EROD assay

Biotransformation enzyme activities, such as Cytochrome P450 1A (CYP1A), have been widely used as biomarkers for organic contaminant exposure (Whyte et al., 2000). A non-destructive EROD assay was modified and optimized from a previous study (Noury et al., 2006). BaP, as a prototype and well documented PAH, was applied as positive control and in toxic equivalency calculation (Nisbet and LaGoy, 1992; ATSDR, 1995). In the current study, exposure of only 1.25% and 2.5% dilutions of HF-FPW-S and HF-FPW-SF resulted in significant induction of EROD activity in zebrafish embryo. Compared to the control group, exposure to HF-FPW-S diluted to 1.25% and 2.5% were equivalent to 203 ± 26 and 393 ± 27 ng/L of BaP, respectively. Exposure of HF-FPW-AC did not cause significant effects on EROD activity in zebrafish embryo. Detailed information is provided in SI. (Fig. S4).

The experiment where we co-exposed BaP and HF-FPWs was designed specifically to investigate PAH-derived induction of EROD activity and also to detect the potential for interaction between different components present in HF-FPW. Traditionally, a synergistic or potentiating effect is defined as the interaction between two well defined chemical signatures. Given the complexity of HF-FPW, a classical demonstration of synergism would not be possible. However, if we view a complex mixture as a single contaminant, then the interaction between a model chemical (e.g BaP) and a complex mixture of contaminants can be used to predict synergism or potentiation. This concurrent exposure method has been applied to various environmental mixtures (Björkblom et al., 2008; He et al., 2011; Adebambo et al., 2015). In this study, a low range of BaP concentrations (from 0 to 1000 ng/L) were co-exposed with 2.5% of HF-FPW-AC, HF-FPW-SF, and HF-FPW-S, respectively, and in parallel, a low range exposure for BaP standard curve (0 to 1000 ng/L) was also conducted (Fig. 4B, Fig. S5). Co-exposure of

2.5% HF-FPW-S resulted in higher induction of EROD activity compared to all other co-exposure and BaP alone groups at all levels of BaP concentrations. Co-exposure of 2.5% HF-FPW-SF resulted in higher induction of EROD activity compared to BaP control groups at all levels of BaP concentrations, and higher induction of EROD activity compared to co-exposure of 2.5% HF-FPW-AC at BaP concentrations of 0, 10, 250, 500, and 1000 ng/L (Fig. 4B). The potential synergistic/additive effects of co-exposure of BaP and HF-FPWs on EROD induction (equivalency; Eq), was calculated based on the following equation:

$$Eq^{\text{synergism}} [\text{HF-FPW} \times \text{BaP}(n)] = Eq[\text{HF-FPW} + \text{BaP}(n)] - Eq[\text{HF-FPW}] - Eq[\text{BaP}(n)]$$

where Eq is the BaP equivalency (ng/L); HF-FPW is the exposed 2.5% of HF-FPW-AC, HF-FPW-SF or HF-FPW-S; BaP(n) is the co-exposed BaP with concentration of n; $\times$ represents the interaction between HF-FPW and BaP; + represents the co-exposure experiment of HF-FPW and BaP.

$Eq^{\text{synergism}}$ values from co-exposure of 2.5% HF-FPW-S with 100, 250, 500, and 1,000 ng/L of BaP were 428 ± 87 , 858 ± 116 , 1017 ± 147 , and 1264 ± 220 ng/L, respectively, which were significantly higher than those of 2.5% HF-FPW-SF and 2.5% HF-FPW-AC co-exposure groups. Co-exposure of 2.5% HF-FPW-SF with BaP also showed general trends toward higher levels of $Eq^{\text{synergism}}$ values compared to 2.5% HF-FPW-AC groups, with significantly higher $Eq^{\text{synergism}}$ values in co-exposure groups of 500 and 1000 ng/L of BaP (257 ± 85 , and 483 ± 133 ng/L, respectively) (Fig. 4C).

Our results strongly suggest that the presence of organic contaminants, especially polycyclic aromatic hydrocarbons (PAHs) in HF-FPW, may be the major contributor of EROD induction. PAHs are a group of chemicals that are formed naturally or anthropogenically during the incomplete combustion of organic substances, including coal, oil gas, wood, garbage,

450 tobacco, etc. (ATSDR, 1995). PAHs are carcinogens and usually highly concentrated in polluted
451 sediments and organic matter due to high lipophilicity (ATSDR, 1995; Hylland, 2006).. EROD
452 activity, which is a catalytic measurement of cytochrome p4501A induction, is a widely
453 used [biomarker](#) in fish [bioassays](#) for the exposure of aryl hydrocarbon receptor agonists,
454 including dioxins, polychlorinated biphenyls (PCBs) and PAHs (Whyte et al., 2000). In the
455 current study, the HF-FPW-S sample contained approximately 2 times more total PAHs and
456 alkylated PAHs than the HF-FPW-SF sample, consistent to the fact that the HF-FPW-S resulted
457 in stronger effect on EROD induction than HF-FPW-SF. Total PAHs in the HF-FPW-AC was
458 below the detection limit, consistent to the fact that HF-FPW-AC did not have any observable
459 effect on EROD activity in zebrafish embryos.

460 The EROD induction levels caused by exposure to HF-FPWs are explainable by the total
461 amounts of PAHs and alkylated PAHs present in the HF-FPWs. Exposure to 2.5% HF-FPW-S
462 resulted in 2.05 ± 0.13 -fold induction of EROD activity, equivalent to 393 ± 27 ng/L of BaP.
463 Therefore, full-strength HF-FPW-S would have a total PAHs level equivalent to 15,720 ng/L of
464 BaP. However, total PAHs and alkylated PAHs in the HF-FPW-S sample was measured to be
465 only ~ 2000 ng/L. A possible explanation for this discrepancy is the presence of unknown
466 chemical(s) in HF-FPWs which is(are) strong AhR agonist(s) and EROD inducer(s). Some
467 environmental contaminants, such as PCBs and dioxin-like compounds, can induce EROD
468 activity both *in vitro* and *in vivo* (Tillitt et al., 1991; Whyte et al., 2000). A second reason for the
469 noted discrepancy in BaP equivalency is that a linear relationship has been applied close to the
470 detection limit. There was a chance that in the lower region of the curve, the regression is non-
471 linear, thus overestimating BaP equivalency. Future study is needed to confirm whether other
472 contaminants with agonistic effects on EROD are present in HF-FPWs. However, the most

likely explanation is that EROD agonistic contaminants such as PAHs act together with the sediment fraction resulting in synergistic induction of EROD activity when compared to the equivalent amount of PAHs alone. This is supported by the co-exposure experiment (Fig. 4C) where EROD induction was equivalent to $1,080 \pm 200$ ng/L BaP in the co-exposure group of 2.5% HF-FPW-S and 1,000 ng/L BaP, neither of which cannot be accounted for by either the exposure of 2.5% HF-FPW-S or 1,000 ng/L BaP alone. We also present evidence that uncharacterized material was observed to accumulate on the surface, particularly near the gill bud region (Fig. 4D V). Cationic polymers are known to be present HF-FPW and bind to gill surface (Kerr et al., 2014) and these might act as a sorbent/delivery vehicle for sediment associated PAHs, enhancing the exposure to these contaminants and resulting in a synergistic effect on EROD activity induction.

4. Conclusions

In this study, we characterized the chemical and toxicological signatures of a real HF-FPW sample from the Devonian-aged Duvernay Formation, Alberta, Canada. Our study demonstrates that HF-FPW is a highly complex mixture with numerous anthropogenic and naturally occurred inorganic and organic contaminants. As suggested here for the conversion of organic phosphates, the possibility that chemical ingredients in HF fluids may be transformed during down-hole reactions must also be considered. Exposure to HF-FPW can induce acute toxicity in zebrafish embryos. Removal of suspended solids, which reduces the total organic contaminant level by approximately 50%, is able to partially mitigate the acute lethal toxicity of HF-FPW. However, the acute toxicity is complicated by a dominant toxicity effect of the high salinity derived from interactions of HF fluid with the target formation during fracturing. Exposure to HF-FPW can

also induce EROD activity in zebrafish embryos. The potential synergistic effect on induction of EROD activity suggests the presence of interactions among the contaminants in HF-FPW and therefore standard chemical risk analysis may be significantly more complex than current protocols for risk management. Extensive future studies are required to further characterize this complex mixture and further link the chemistry to the measured toxicity (eg. the toxicity and mechanism(s) of the organic fractions of HF-FPW). Ultimately, the goal is to develop proper risk assessment protocols for surficial spills, to inform various stakeholders on required mitigation and hazard assessment protocols, and to inform producers thereby allowing them to undertake mitigation efforts to reduce the environmental impacts of HF-FPW spills.

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Appendix A. Supporting information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

REFERENCES

518 Adebambo, O.A.; Ray, P.D.; Shea, D.; Fry, R.C., 2015. Toxicological responses of
 519 environmental mixtures: Environmental metal mixtures display synergistic induction of
 520 metal-responsive and oxidative stress genes in placental cells. *Toxicol. Appl. Pharmacol.*,
 521 289(3), 534-541.
 522 Agency for Toxic Substances and Disease Registry (ATSDR), 1995. Toxicological profile for
 523 Polycyclic Aromatic Hydrocarbons (PAHs) (Report). Atlanta, GA: U.S. Department of
 524 Health and Human Services, Public Health Service. Retrieved 2016-09-30.
 525 Alberta Energy Regulator (AER), 2016. Compliance Dashboard. Available at:
 526 <http://www1.aer.ca/compliancedashboard/index.html> Retrieved 2016-09-30.
 527 Alessi, D.S.; Zolfaghari, A.; Kletke, S.; Gehman, J.; Allen, D.M.; Goss, G.G., 2016.
 528 Comparative analysis of hydraulic fracturing wastewater practices in unconventional
 529 shale development: water sourcing, treatment, and disposal practices. *Can. Water Resour.*
 530 *J.* in press, doi: 10.1080/07011784.2016.1238782.
 531 Allan, S.E., Smith, B.W., Anderson, K.A., 2012. Impact of the deepwater horizon oil spill on
 532 bioavailable polycyclic aromatic hydrocarbons in Gulf of Mexico coastal waters. *Environ.*
 533 *Sci. Technol.* 46(4), 2033-2039.
 534 Anfort, S.J., Bachu, S., Bentley, L.R., 2001. Regional-scale hydrogeology of the upper
 535 Devonian-lower Cretaceous sedimentary succession, south-central Alberta basin, Canada.
 536 *AAPG Bulletin.* 85(4), 637-660. Aryal, R., Furumai, H., Nakajima, F., Beecham, S., 2013.
 537 Variation in PAH patterns in road runoff. *Water Sci. Technol.* 67(12), 2699-2705.
 538 Björklom, C.; Salste, L.; Katsiadaki, I.; Wiklund, T.; Kronberg, L., 2008. Detection of
 539 estrogenic activity in municipal wastewater effluent using primary cell cultures from
 540 three-spined stickleback and chemical analysis. *Chemosphere.* 73(7), 1064-1070.

541 Colborn, T., Kwiatkowski, C., Schultz, K., Bachran, M., 2011. Natural Gas Operations from a
542 Public Health Perspective. *Hum. Ecol. Risk Assess.* 17(5), 1039-1056.

543 Darrah, T.H., Vengosh, A., Jackson, R.B., Warner, N.R., Poreda, R.J., 2014. Noble gases
544 identify the mechanisms of fugitive gas contamination in drinking-water wells overlying
545 the Marcellus and Barnett Shales. *Proc. Natl. Acad. Sci. USA.* 111(39), 14076-14081.

546 DiGiulio, D.C., Jackson, R.B., 2016. Impact to Underground Sources of Drinking Water and
547 Domestic Wells from Production Well Stimulation and Completion Practices in the
548 Pavillion, Wyoming, Field. *Environ. Sci. Technol.* 50(8), 4524-4536.

549 Drollette, B.D., Hoelzer, K., Warner, N.R., Darrah, T.H., Karatum, O., O'Connor, M.P., Nelson,
550 R.K., Fernandez, L.A., Reddy, C.M., Vengosh, A., Jackson, R.B., Elsner, M., Plata, D.L.,
551 2015. Elevated levels of diesel range organic compounds in groundwater near Marcellus
552 gas operations are derived from surface activities. *Proc. Natl. Acad. Sci. USA.* 112(43),
553 13184-13189.

554 Dunn, L., Schmidt, G., Hammermaster, K., Brown, M., Bernard, R., Wen, E., Befus, R.,
555 Gardiner, S., 2012. The Duvernay Formation (Devonian): Sedimentology and Reservoir
556 Characterization of a Shale Gas/Liquids play in Alberta, Canada. In: *GeoConvention*,
557 Canadian Society of Petroleum Geologists Annual Core Convention program with
558 abstracts.

559 Elliott, E.G., Ettinger, A.S., Leaderer, B.P., Bracken, M.B., Deziel, N.C., 2016. A systematic
560 evaluation of chemicals in hydraulic-fracturing fluids and wastewater for reproductive
561 and developmental toxicity. *J. Expo. Sci. Environ. Epidemiol.* , 1-10.

562 Environment Agency, UK. (UKEA), 2009. An overview of the environmental risk evaluation
563 reports for aryl phosphate esters. Available at:

564 [https://www.gov.uk/government/publications/an-overview-of-the-environmental-risk-](https://www.gov.uk/government/publications/an-overview-of-the-environmental-risk-evaluation-reports-for-aryl-phosphate-esters)
565 [evaluation-reports-for-aryl-phosphate-esters](https://www.gov.uk/government/publications/an-overview-of-the-environmental-risk-evaluation-reports-for-aryl-phosphate-esters) Retrieved 2016-09-30.

566 Environmental Protection Agency, USA. (USEPA), 2002. Methods for Measuring the Acute
567 Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Test
568 Method 2021.0. 5th Edition.

569 Environmental Protection Agency, USA. (USEPA), 2015. Hydraulic Fracturing for Oil and Gas
570 and Its Potential Impact on Drinking Water Resources: Chemicals Identified in Hydraulic
571 Fracturing Fluids and/or Flowback and Produced Water. Available at:
572 <http://www.epa.gov/hfstudy> Retrieved 2016-09-30.

573 Frac Focus, 2016. What chemicals are used. Available at: [http://fracfocus.ca/chemical-use/what-](http://fracfocus.ca/chemical-use/what-chemicals-are-used)
574 [chemicals-are-used](http://fracfocus.ca/chemical-use/what-chemicals-are-used) Retrieved 2016-09-30.

575 Goss, G.G., 2015. Unconventional Wastewater Management: A comparative review and analysis
576 of hydraulic fracturing wastewater management practices across four North American
577 basins. Canadian Water Network. Available at: [http://www.cwn-rce.ca/project-](http://www.cwn-rce.ca/project-library/project/unconventional-wastewater-management-a-comparative-review-and-analysis-of-hydraulic-fracturing-wastewater-management-practices-across-four-north-american-basins?u=category%3Dhydraulic-fracturing)
578 [library/project/unconventional-wastewater-management-a-comparative-review-and-](http://www.cwn-rce.ca/project-library/project/unconventional-wastewater-management-a-comparative-review-and-analysis-of-hydraulic-fracturing-wastewater-management-practices-across-four-north-american-basins?u=category%3Dhydraulic-fracturing)
579 [analysis-of-hydraulic-fracturing-wastewater-management-practices-across-four-north-](http://www.cwn-rce.ca/project-library/project/unconventional-wastewater-management-a-comparative-review-and-analysis-of-hydraulic-fracturing-wastewater-management-practices-across-four-north-american-basins?u=category%3Dhydraulic-fracturing)
580 [american-basins?u=category%3Dhydraulic-fracturing](http://www.cwn-rce.ca/project-library/project/unconventional-wastewater-management-a-comparative-review-and-analysis-of-hydraulic-fracturing-wastewater-management-practices-across-four-north-american-basins?u=category%3Dhydraulic-fracturing) Retrieved 2016-09-30.

581 Hammond, M., Nunn, H., Rogers, G., Lee, H., Marghitoiu, A.L., Perez, L., Nashed-Samuel, Y.,
582 Anderson, C., Vandiver, M., Kline, S., 2013. Identification of a leachable compound
583 detrimental to cell growth in single-use bioprocess containers. PDA J. Pharm. Sci.
584 Technol. 67(2), 123-134.

585 Harkness, J.S., Dwyer, G.S., Warner, N.R., Parker, K.M., Mitch, W.A., Vengosh, A., 2015.
586 Iodide, bromide, and ammonium in hydraulic fracturing and oil and gas wastewaters:
587 environmental implications. *Environ. Sci. Technol.* 49(3), 1955-1963.

588 He, Y., Wiseman, S.B., Hecker, M., Zhang, X., Wang, N., Perez, L.A., Jones, P.D., El-Din,
589 M.G., Martin, J.W., Giesy, J.P., 2011. Effect of ozonation on the estrogenicity and
590 androgenicity of oil sands process-affected water. *Environ. Sci. Technol.* 45(15), 6268-
591 6274.

592 He, Y., Patterson, S., Wang, N., Hecker, M., Martin, J.W., El-Din, M.G., Giesy, J.P., Wiseman,
593 S.B., 2012a. Toxicity of untreated and ozone-treated oil sands process-affected water
594 (OSPW) to early life stages of the fathead minnow (*Pimephales promelas*). *Water Res.*
595 46(19), 6359-6368.

596 He, Y., Wiseman, S.B., Wang, N., Perez-Estrada, L.A., El-Din, M.G., Martin, J.W., Giesy, J.P.,
597 2012b. Transcriptional responses of the brain-gonad-liver axis of fathead minnows
598 exposed to untreated and ozone-treated oil sands process-affected water. *Environ. Sci.*
599 *Technol.* 46(17), 9701-9708.

600 Hylland, K., 2006. Polycyclic aromatic hydrocarbon (PAH) ecotoxicology in marine ecosystems.
601 *J. Toxicol. Environ. Health A.* 69(1-2), 109-123.

602 Incardona, J.P., Gardner, L.D., Linbo, T.L., Brown, T.L., Esbaugh, A.J., Mager, E.M., Stieglitz,
603 J.D., French, B.L., Labenia, J.S., Laetz, C.A., Tagal, M., Sloan, C.A., Elizur, A., Benetti,
604 D.D., Grosell, M., Block, B.A., Scholz, N.L., 2014. Deepwater Horizon crude oil impacts
605 the developing hearts of large predatory pelagic fish. *Proc. Natl. Acad. Sci. USA.*
606 111(15), E1510-1518.

607 Isales, G.M., Hipszer, R.A., Raftery, T.D., Chen, A., Stapleton, H.M., Volz, D.C., 2015.
 608 Triphenyl phosphate-induced developmental toxicity in zebrafish: Potential role of the
 609 retinoic acid receptor. *Aquat. Toxicol.* 161, 221-230
 610 Jackson, R.B., Vengosh, A., Darrah, T.H., Warner, N.R., Down, A., Poreda, R.J., Osborn, S.G.,
 611 Zhao, K., Karr, J.D., 2013. Increased stray gas abundance in a subset of drinking water
 612 wells near Marcellus shale gas extraction. *Proc. Natl. Acad. Sci. USA.* 110(28), 11250-
 613 11255.
 614 Kahrilas, G.A., Blotevogel, J., Stewart, P.S., Borch, T., 2015. Biocides in hydraulic fracturing
 615 fluids: a critical review of their usage, mobility, degradation, and toxicity. *Environ. Sci.*
 616 *Technol.* 49(1), 16-32.
 617 Kalmykova, Y., Björklund, K., Strömvall, A.M., Blom, L., 2013. Partitioning of polycyclic
 618 aromatic hydrocarbons, alkylphenols, bisphenol A and phthalates in landfill leachates and
 619 stormwater. *Water Res.* 47(3), 1317-1328.
 620 Kelly, E.N., Short, J.W., Schindler, D.W., Hodson, P.V., Ma, M., Kwan, A.K., Fortin, B.L., 2009.
 621 Oil sands development contributes polycyclic aromatic compounds to the Athabasca
 622 River and its tributaries. *Proc. Natl. Acad. Sci. USA.* 106(52), 22346-22351.
 623 Kerr, J.L., Lumsden, J.S., Russell, S.K., Jasinska, E.J., Goss, G.G., 2014. Effects of anionic
 624 polyacrylamide products on gill histopathology in juvenile rainbow trout (*Oncorhynchus*
 625 *mykiss*). *Environ. Toxicol. Chem.* 33(7), 1552-1562.
 626 Lauer, N.E., Harkness, J.S., Vengosh, A., 2016. Brine Spills Associated with Unconventional Oil
 627 Development in North Dakota. *Environ. Sci. Technol.* 50(10), 5389-5397.

628 Lester, Y., Ferrer, I., Thurman, E.M., Sitterley, K.A., Korak, J.A., Aiken, G., Linden, K.G., 2015.
 629 Characterization of hydraulic fracturing flowback water in Colorado: implications for
 630 water treatment. *Sci. Total Environ.* 512-513, 637-644.

631 Llewellyn, G.T., Dorman, F., Westland, J.L., Yoxtheimer, D., Grieve, P., Sowers, T., Humston-
 632 Fulmer, E., Brantley, S.L., 2015. Evaluating a groundwater supply contamination
 633 incident attributed to Marcellus Shale gas development. *Proc. Natl. Acad. Sci. USA.*
 634 112(20), 6325-6330.

635 Martin, J.W., Han, X., Peru, K.M., Headley, J.V., 2008. Comparison of high- and low-resolution
 636 electrospray ionization mass spectrometry for the analysis of naphthenic acid mixtures in
 637 oil sands process water. *Rapid Commun. Mass Spectrom.* 22(12), 1919-1924.

638 Martin, J.W., Barri, T., Han, X., Fedorak, P.M., El-Din, M.G., Perez, L., Scott, A.C., Jiang, J.T.,
 639 2010. Ozonation of oil sands process-affected water accelerates microbial
 640 bioremediation. *Environ. Sci. Technol.* 44(21), 8350-8356.

641 Mayes, M.W., Potter, H.A., Jarvis, A.P., 2009. Novel approach to zinc removal from circum-
 642 neutral mine waters using pelletised recovered hydrous ferric oxide. *J. Hazard. Mater.*
 643 162(1), 512-520.

644 Nielsen, K., Kalmykova, Y., Strömvall, A.M., Baun, A., Eriksson, E., 2015. Particle phase
 645 distribution of polycyclic aromatic hydrocarbons in stormwater- Using humic acid and
 646 iron nano-sized colloids as test particles. *Sci. Total Environ.* 532, 103-111.

647 Nisbet, I.C., LaGoy, P.K., 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic
 648 hydrocarbons (PAHs). *Regul. Toxicol. Pharmacol.* 16(3), 290-300.

649 Noury, P., Geffard, O., Tutundjian, R., Garric, J., 2006. Non-destructive in vivo measurement of
 650 ethoxyresorufin biotransformation by zebrafish prolarva: development and application.
 651 Environ. Toxicol. 21(4), 324-331.

652 Orem, W., Tabu, C., Varonka, M., Lerch, H., Bates, A., Engle, M., Crosby, L., McIntosh, J.,
 653 2014. Organic substances in produced and formation water from unconventional natural
 654 gas extraction in coal and shale. Int. J. Coal Geol. 126(1), 20-31.

655 Osborn, S.G., Vengosh, A., Warner, N.R., Jackson, R.B., 2011. Methane contamination of
 656 drinking water accompanying gas-well drilling and hydraulic fracturing. Proc. Natl.
 657 Acad. Sci. USA. 108(20), 8172-8176.

658 Rowland, S.J., West, C.E., Jones, D., Scarlett, A.G., Frank, R.A., Hewitt, L.M., 2011. Steroidal
 659 aromatic 'naphthenic acids' in oil sands process-affected water: structural comparisons
 660 with environmental estrogens. Environ. Sci. Technol. 45(22), 9806-9815.

661 Sang, W., Stoof, C.R., Zhang, W., Morales, V.L., Gao, B., Kay, R.W., Liu, L., Zhang, Y.,
 662 Steenhuis, T.S., 2014. Effect of hydrofracking fluid on colloid transport in the
 663 unsaturated zone. Environ. Sci. Technol. 48(14), 8266-8274.

664 Stringfellow, W.T., Domen, J.K., Camarillo, M.K., Sandelin, W.L., Borglin, S., 2014. Physical,
 665 chemical, and biological characteristics of compounds used in hydraulic fracturing. J.
 666 Hazard. Mater. 275, 37-54.

667 Thurman, E.M., Ferrer, I., Rosenblum, J., Linden, K., Ryan, J.N., 2016. Identification of
 668 polypropylene glycols and polyethylene glycol carboxylates in flowback and produced
 669 water from hydraulic fracturing. J Hazard Mater. pii:S0304-3894(16) 30168-6.

670 Tillitt, D.E., Ankley, G.T., Verbrugge, D.A., Giesy, J.P., Ludwig, J.P., Kubiak, T.J., 1991. H4IIE
 671 rat hepatoma cell bioassay-derived 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in

672 colonial fish-eating waterbird eggs from the Great Lakes. Arch. Environ. Contam.
 673 Toxicol. 21(1), 91-101.

674 Vengosh, A., Jackson, R.B., Warner, N., Darrah, T.H., Kondash, A., 2014. A critical review of
 675 the risks to water resources from unconventional shale gas development and hydraulic
 676 fracturing in the United States. Environ. Sci. Technol. 48(15), 8334-8348.

677 Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD)
 678 activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. 30(4), 347-570.

679 Zachara, J.M., Smith, S.C., 1994. Edge site contributions to Cd sorption on specimen and soil-
 680 derived smectite in Na⁺ and Ca²⁺ electrolytes. Soil Sci. Soc. Am. J. 57, 1491-1501.

681 Zhang, Q., Pan, B., Zhang, W., Pan, B., Zhang, Q., Ren, H., 2008. Arsenate removal from
 682 aqueous media by nanosized hydrated ferric oxide (HFO)-loaded polymeric sorbents:
 683 Effect of HFO loadings. Ind. Eng. Chem. Res. 47(11), 3957-3962.

684

685 **TABLE**

686 **Table 1.** Selected physiochemical and elemental parameters of the tested HF-FPW sample.

687

7-day HF-FPW Sample (pH = 4.78)			
Parameters	Method	Mean Conc. (mg/L)	<u>% Deviation</u>
TDS	Evaporation	243,000 2,624	<u>0.5</u>
TN	TC/TN	498	<u>0.92</u>
	Analyzer		
TC	TC/TN	211	<u>1.9</u>
	Analyzer		
Na	ICP-MS/MS	70,000	<u>6.7</u>
Ca	ICP-MS/MS	11,800	<u>2.2</u>
K	ICP-MS/MS	2,570	<u>6.1</u>
Cl	IC	136,000	<u>8.4</u>

688

Formatted Table

FIGURES

No color should be used for any figures in print.

Fig. 1. A) SEM image of HF-FPW sediments and solids showing a variety of morphologies. B) A higher magnification SEM image showing spherical particles. C) EDX profiles of spherical particles with the grey block representing the exterior of a sphere found in Panel A marked with plaid square and the white blocks from the interior of a sphere marked with strip square in Panel B.

Commented [sf2]: The labels are switched in the EDX elemental analysis plot Fig 1 C. The carbon should higher in the interior not the exterior, the labels just need to be switched.

Fig. 2. Untargeted analysis of HF-FPW sample by HPLC-Orbitrap MS. (A) Major species in a 3-dimensional view of retention time, m/z , and intensity, (B) each detectable species with a distinct empirical chemical formula plotted by Kendrick mass and Kendrick mass defect (*Kendrick Plot*), (C) an extracted ion chromatogram of three confirmed phosphate species ($C_xH_yPO_4$). The molecular formulas of phosphates (#1: triphenyl phosphate; #2: bis(2,4-di-tert-butylphenyl) phosphate and #3: tris(2,4-di-tert-butylphenyl) phosphate. Example MS/MS spectra for compounds #2 and #3 are shown in SI (Fig. S3).

Fig. 3. Parent PAHs, alkyl PAHs, dibenzothiophene (DBT) and retene in raw (HF-FPW-S) and sediment-free (HF-FPW-SF) samples.

Fig. 4. Exposure to HF-FPW resulted in adverse effects on zebrafish embryo/larvae. AC represents HF-FPW-AC. SF represents HF-FPW-SF. S represents HF-FPW-S. A) Mortality of zebrafish embryo exposed to HF-FPWs from 1 to 96 hpf (n=6), and from 24 to 96 hpf. B) EROD activity induced by co-exposure of HF-FPW in zebrafish embryos. Different letters represent statistical differences within the co-exposure groups with the same BaP concentration. C) The synergistic inducing levels of EROD activity resulted from co-exposure of HF-FPWs in zebrafish embryos. * represents the significant differences of synergistic inducing level of EROD between HF-FPW-S co-exposure group and HF-FPW-AC co-exposure group (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). * represents the significant differences of synergistic inducing level of EROD between HF-FPW-S co-exposure group and HF-FPW-SF co-exposure group (** $p < 0.01$, * $p < 0.05$). * represents the significant differences of synergistic inducing level of EROD between HF-FPW-SF co-exposure group and HF-FPW-AC co-exposure group ($p < 0.01$, $p < 0.05$). D) Suspended solids in HF-FPWs aggregated and adhered on the surface of embryo chorion. Pictures ($\times 40$) were taken at 48 hpf after 24 h exposure of HF-FPWs. I represents the embryo in control group. II represents the embryo in 2.5% of HF-FPW-AC group. III represents the embryo in 2.5% of HF-FPW-SF group. IV represents the embryo in 2.5% of HF-FPW-S group. V represents unidentified material(s) aggregated on body surface of larvae. Arrow points out the exact position of interest.

SUPPORTING INFORMATION

1. Materials and methods

1.1. Chemicals

Benzo[a]Pyrene (BaP, CAS No. 50-32-8), triphenyl phosphate, diphenyl phosphate, bis(2,4-di-tert-butylphenyl) phosphate, tris(2,4-di-tert-butylphenyl) phosphate were purchased from Sigma-Aldrich (Ontario, Canada). Tris(2,4-ditert-butylphenyl) phosphite was purchased from Toronto Research Chemicals (Ontario, Canada). 7-Ethoxyresorufin (7-ER, CAS No. 5725-91-7) was purchased from Cayman Chemical (Michigan, USA). Twenty-three parent PAHs standards (unlabelled) and 16 internal standards (deuterium-labelled) were purchased from Wellington Lab, 15 alkyl PAHs standards were purchased from Norway Chiron, and retene standards was purchased from ChemService in 2013.

1.2. Dilution water

Dilution water was prepared with nano-pure water (generated by PURELAB Flex, ELGA LabWater) and reagent grade salts according to the guidelines of preparation of moderately hard water by US EPA, which was prepared in 20 L batches containing 1.92 g of NaHCO₃, 1.2 g of CaSO₄·2H₂O, 1.2 g of MgSO₄, and 0.08 g of KCl. The resulting dilution water was kept at 25±1 °C and aerated prior to use. BaP and 7-ER were dissolved in absolute ethanol, and then added to dilution water for exposure. The solvent concentration never exceeded 0.05% (v/v) during the entire experiment.

1.3 Zebrafish husbandry

762 Adult zebrafish were cultured under standardized conditions in the aquatic facility in the
763 Department of Biological Sciences, University of Alberta and all experiments conducted under
764 University of Alberta animal use protocol 00001334. Embryos were obtained from matured fish
765 bred from two male and one female fish, placed separately in a breeding aquarium. Fertilized
766 embryos from six to eight breeding aquaria were collected, rinsed and pooled for further
767 experiments. For the exposure using 1 h post fertilization (hpf) embryos, the embryos were
768 randomly selected and placed into exposure chambers immediately. For the exposure using 24
769 hpf embryos, the embryos were placed in petri dish (around 100 embryos per plate) containing
770 15 mL of dilution water. Any undeveloped embryos were removed during the following 24
771 hours. After that, the developing embryos were randomly selected and placed into exposure
772 chambers to initiate the exposure at 25 ± 1 °C under 16h/8h light/dark cycle.

773

774 **1.4. Ion Chromatography**

775 HF-FPW samples for anion (Br^- , Cl^- , SO_4^{2-}) analysis were first filtered through a 0.2 μm nylon
776 membrane and then analyzed at the University of Alberta Natural Resources Analytical Lab
777 (NRAL) using a Dionex Ion chromatography DX 600 with 4mm analytical column AS9-HC,
778 guard column AG9-HC, and a 4 mm ASRS Ultra suppressor.

779

780 **1.5. Total Organic Carbon and Total Nitrogen**

781 Samples for total organic carbon (TOC) and total nitrogen (TN) analysis were prefiltered using a
782 0.2 μm nylon membrane before being analyzed. Analysis was performed at the NRAL using a
783 Shimadzu TOC-V CHS/CSN Model TOC Analyzer. TOC was measured as non-purgeable
784 organic carbon.

785
786 **1.6. Inductively Coupled Plasma-Mass Spectroscopy**
787 HF-FPW samples for dissolved metal analysis were first passed through a 0.2 µm nylon
788 membrane for the removal of solids. The HF-FPW sample were then diluted using 2% trace
789 metal grade nitric acid and 0.5% trace metal grade hydrochloric acid prepared with 18MΩ
790 ultrapure water. A table of dilutions used for each element can be found in Table S1. All analysis
791 for cations and Br were performed using an Agilent 8800 Triple Quadrupole ICP-MS (ICP-
792 MS/MS) at an RF power of 1550 W and a RF reflected power of 18 W. The ICP-MS/MS was
793 operated with a microMist nebulizer and nickel/copper cones. Several unique features of the
794 ICP-MS/MS were utilized during analysis including high matrix mode with 8 mL/min argon
795 dilution, MS/MS mode for greater mass resolution and the collision/reaction cell with He gas.
796 Due to the complex nature of the brine a standard addition method was used for the
797 determination of all metals and to match matrix of HF-FPW. The elemental analyses for each
798 element from three runs were completed to determine the error in measurement. Full operating
799 conditions for each analysis run can be found in Table S2.

800

801 **2. Results and discussion**

802 **2.1. Inorganic analysis**

803 The pH of the 7 day HF-FPW sample was measured to be 4.78 and the TDS determined through
804 evaporation was found to be ~~242.624~~ 242.3 mg/L ± 0.54%. The SS were found to be ~~22.549~~ 22.1 mg/L
805 ±5.0% and the TS were found to be 403 mg/L ±13%.

806

807 **2.2. Ion Chromatography**

808 Ion chromatography (IC) was used to determine the chloride (Cl^-), bromide (Br^-), and sulfate
809 (SO_4^{2-}) concentrations. Chloride was measured to be 136,000 mg/L which is approximately 56%
810 of the total TDS and the primary anion of the HF-FPW sample. The bromide and sulfate were
811 measured to be 247 mg/L and 48.7 mg/L, respectively. Full results of elemental analysis can be
812 found in Table S1.

813

814 **2.3. Total Organic Carbon and Nitrogen**

815 The total organic carbon (TOC) of the HF-FPW sample was measured to be 212 mg/L or
816 approximately 0.087% of the total TDS. This measurement only quantifies the dissolved organic
817 carbon molecules, additionally organics maybe associated with solid phases. TOC measurements
818 may have been low as a thinly film was observed on the surface of the bulk HF-FPW sample as
819 delivered from the field. It is possible that the sampling probe was below the surface of this film
820 and therefore missed an immiscible fraction of the TOC associated with the sample. The total
821 nitrogen (TN) was found to be 498 mg/L or 0.21% of the total TDS of the HF-FPW sample.

822

823 **2.4. ICP-MS/MS**

824 An ICP-MS/MS (Agilent 8800) was used to measure the concentration of 14 elements (Table
825 S1). In all cases the deviation in replicate analysis was under 10%. The cation with the greatest
826 concentration was found to be Na which found to be 70,000 mg/L or 28.8% of the total TDS of
827 the sample. Only Ca and K were also found to make up more than 1% of the TDS, 11,800 mg/L
828 and 4.85%, and 2,570 mg/L and 1.05%, respectively. Other major cations (operationally defined
829 as >100 mg/L here) included Mg and Sr; a complete table of the elements and their
830 concentrations can be found in Table S1.

Thirteen of the elements measured by ICP-MS/MS were cations, with bromide being the sole anion. The bromide concentration measure by ICP-MS/MS was measured to be 276 mg/L, a value about 10% greater than the concentration measured by IC. In all 223,960 mg/L of the total 242,624 mg/L TDS have been accounted for or 92.3%, well within 10% of the total TDS. Since only TOC was measured, a portion of the missing TDS is likely comprised of additional elements including, H, O, and P which would be components of the larger organic molecules to which the TOC belongs. A charge balance of the solution is shown in Table S2. There was found to be a total of 3825.8 meq of cations and -3836.7 meq of anions, a difference of -11 meq or only 0.28% deviation between the cations and anions, indicating that the charged species in solution are well characterized.

841

842 2.5. SEM/EDX analysis

Fig. S1 shows three additional types of particles found in HF-FPW suspended solids with differing morphologies and compositions, and which make up a smaller overall fraction of the suspended solids mass. Fig. 4A depicts a barium/strontium sulfate crystal with a radial, needle-like morphology. The carbon in the EDX elemental analysis is likely from the carbon sputter coat. The next most abundant elements were oxygen (41%, ± 1.3), sulfur (8.7%, ± 0.04), barium (6.2%, ± 0.06) and strontium (2.3%, ± 0.008). The molar percentages are close to the stoichiometry for Ba/SrSO₄, strongly suggesting that this phase is indeed barium/strontium sulfate. Fig. 4C. shows a silicate particle which is mainly composed of oxygen (29.7%, ± 1.9), silica (6.7%, ± 0.23), and sodium (2.5%, ± 0.03), with lesser amounts ($<1\%$, ± 0.002) of calcium, potassium, iron, magnesium, and aluminum. Fig. 4E shows an aluminosilicate with an elemental composition of 47.2% (± 2.6) oxygen, 13.3% (± 0.12) silica, 5.0% (± 0.02) aluminum, 3.7%

854 (±0.01) potassium, and trace iron and magnesium. These particles are found to a lesser extent
855 than the amorphous iron precipitates and spheres coated with precipitate, and could have two
856 sources: sand used as the proppant during the hydraulic fracturing, or particles broken free from
857 the fractured formation in the subsurface which were transported to the surface with the HF-
858 FPW. Further work is required to determine the origins of these three particle types and the
859 organics and heavy metals that may be sorbed to them.

860

861

862 **Table S1.** Elemental analysis of HF-FPW sample

Element	Isotope	Method	Mean		US EPA
			Concentration (Mg/L)	% Deviation*	MCLs (mg/L)
Na	23	ICP-MS/MS	70,000	6.7	
Li	7	ICP-MS/MS	54.6	4.0	
Mg	24,25	ICP-MS/MS	111	2.0	
Ca	44	ICP-MS/MS	11,800	2.2	
Sr	88	ICP-MS/MS	1,470	2.3	
K	39	ICP-MS/MS	2,570	6.1	
B	10	ICP-MS/MS	71.6	5.8	
Fe	56	ICP-MS/MS	43.1	3.8	0.3 [#]
Zn	64,66,68	ICP-MS/MS	4.41	3.9	5.0 [#]
Pb	206, 207,208	ICP-MS/MS	0.05	3.6	0.015
Ba	137	ICP-MS/MS	5.85	3.2	2.0
Mn	55	ICP-MS/MS	15.8	5.6	0.05 [#]
Th	205	ICP-MS/MS	0.152	0.7	0.002
Cl		IC	136,000	<u>8.4</u>	1.0 [#]
Br	79	IC and ICP-MS/MS	276	5.9	
SO4		IC	4.8	<u>6.0</u>	250 [#]
TN		TC/TN Analyzer	498	<u>0.92</u>	
TC		TC/TN Analyzer	211	<u>1.9</u>	

863 *deviation calculated from three sample replicate runs on ICP-MS/MS

864 [#]Secondary drinking water standards

865

866

867 **Table S2.** Charge Balance of HF-FPW Sample

Element	meq
Na ⁺	3,040
Li ⁺	7.87
Mg ²⁺	91.2
Ca ²⁺	588
Sr ²⁺	33.6
K ⁺	65.8
Fe ³⁺	2.315
Zn ²⁺	0.135
Pb ²⁺	0.0005
Ba ²⁺	0.0851
Mn ²⁺	0.574
Tl ³⁺	0.0007
Cl ⁻	-3,830
Br ⁻	-3.45
SO ₄ ²⁻	-0.15
B [#]	-6.62

868 ⁺As the HF-FPW sample was stored in with headspace and repeatedly exposed to the atmosphere
869 all iron was assumed to be oxidized and to be Fe³⁺.

870 [#]The most common boron species under HF-FPW sample condition would be H₂BO₃⁻.

871

872 **Table S3.** Analyses of PAHs and alkylated PAHs in HF-FPW-S and HF-FPW-SF.

Species	Concentration (ng/L)	
	HF-FPW-S	HF-FPW-SF
Naphthalene*	42.8	56.3
Acenaphthlene*	5.5	13.7
Acenaphthene*	11.1	28.0
Fluorene*	294.4	545.3
Phenanthrene*	79.2	145.3
Anthracene*	22.6	50.7
Fluoranthene*	4.0	9.6
Pyrene*	16.9	37.3
Benz[a]anthracene*	10.2	15.9
Chrysene*	22.5	50.2
Benzo[b]fluoranthene*	4.7	10.3
Benzo[k+j]fluoranthene*	1.1	3.5
Benzo[a]pyrene*	5.8	11.2
Indeno[1,2,3-cd]pyrene*	ND	ND
Benzo[g,h,i]perylene*	ND	ND
Dibenz[a,h]anthracene*	ND	ND
Dibenzothiophene	10.9	23.7
Retene**	22.3	33.6
1-Methylnaphthalene**	52.3	111.1
1-Methylfluorene**	131.3	297.2
1-Methylphenanthrene**	170.2	233.6
3,6-Dimethylephenanthrene**	224.5	333.8
1-Methylpyrene**	26.8	47.7
Total PAHs (EPA 16 PAHs)	520	977
Total PAHs (with alkylated)	1159	2058

873 *USEPA 16 PAHs. **Alkylated PAHs.

874

Fig. S1. SEM images and corresponding EDX-determined elemental compositions for secondary particle morphologies found in the HF-FPW sample. A. Ba/Sr sulfate particle and B. corresponding EDX for spot 3. C. Silicate particle and D. corresponding EDX for spot 10. E. An aluminosilicate particle and F. corresponding EDX for spot 8.

Fig. S2. Kendrick mass defect in range of 0.05-0.1 and points having same defect in the Kendrick plot potentially corresponding to a distinct chemical species with possible formula, but not structure.

Fig. S3. MS/MS spectral interpretation for compounds #2 (A) and #3 (B).

Fig. S4. A) EROD activity in zebrafish embryo exposed to 1.25% and 2.5% dilutions of HF-FPWs (n=6). Different letters represent significantly different among the entire groups of exposure. AC represents HF-FPW-AC. SF represents HF-FPW-SF. S represents HF-FPW-S. B) The standard curve of fold change of EROD activity relative to control group vs. BaP concentrations ranging from 0 to 10,000 ng/L (n=6).

Fig. S5. The standard curve of fold change of EROD activity relative to control group vs. BaP concentrations ranging from 0 to 1,000 ng/L (n=6). This low range standard curve was generated from the exposure conducted at the same time with co-exposure of HF-FPWs.