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Application of the Target Lipid Model and Passive Samplers to Characterize the Toxicity of Bioavailable Organics in Oil Sands Process-Affected Water

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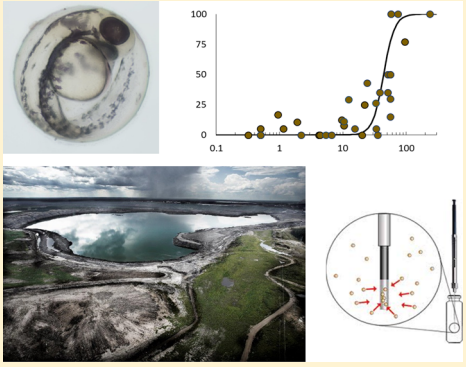
Supporting Information

ABSTRACT: Oil sand operations in Alberta, Canada will eventually include returning treated process-affected waters to the environment. Organic constituents in oil sand process-affected water (OSPW) represent complex mixtures of nonionic and ionic (e.g., naphthenic acids) compounds, and compositions can vary spatially and temporally, which has impeded development of water quality benchmarks. To address this challenge, it was hypothesized that solid phase microextraction fibers coated with polydimethylsiloxane (PDMS) could be used as a biomimetic extraction (BE) to measure bioavailable organics in OSPW. Organic constituents of OSPW were assumed to contribute additively to toxicity, and partitioning to PDMS was assumed to be predictive of accumulation in target lipids, which were the presumed site of action. This method was tested using toxicity data for individual model compounds, defined mixtures, and organic mixtures extracted from OSPW. Toxicity was correlated with BE data, which supports the use of this method in hazard assessments of acute lethality to aquatic organisms. A species sensitivity distribution (SSD), based on target lipid model and BE values, was similar to SSDs based on residues in tissues for both nonionic and ionic organics. BE was shown to be an analytical tool that accounts for bioaccumulation of organic compound mixtures from which toxicity can be predicted, with the potential to aid in the development of water quality guidelines.



INTRODUCTION

Water quality benchmarks are needed to establish acceptable effluent discharge limits that protect aquatic life from the potential effects of treated oil sands process-affected water (OSPW). Organic chemicals found in OSPW include naphthenic acids

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(NAs)[1] but also include a variety of other aromatic, aliphatic, and mixed cyclic compounds as well as those containing heteroatoms such as N and S.[1−9] Recent studies have confirmed that NAs are a major contributor to the toxicity of OSPW.[3,10−13] At present, OSPW is not discharged to the environment because it is recycled back to the extraction process, which, in addition to the lack of water quality benchmarks for the organic constituents in OSPW,[14−17] has led to storage of substantial volumes (~170 000 m^3) on-site in tailings ponds.[18] The practice of impounding process water is not considered sustainable,[17,19] and the eventual return of treated water from mining operations is considered one of the top research priorities for oil sands operators.

One important water quality consideration is that the organic constituents in OSPW exist as complex mixtures, the compositions of which vary by source and producer, as well as spatially and temporally within tailings.[1,20−22] The challenges inherent in assessing these complex mixtures are similar to those of polycyclic aromatic hydrocarbons (PAH) and related petroleum hydrocarbons, which also occur as mixtures that can change as a function of source and time. Since these classes of compounds are widely studied,[23,24] there are opportunities to leverage the lessons learned from research into mixtures of hydrocarbons, which includes application of a modeling framework in combination with passive samplers to measure bioavailability.[24,25]

Models, such as the target lipid model (TLM),[26,27] have been developed to estimate potential hazards of constituents based on molecular structures. This modeling framework has been expanded to several classes of polar[28] and nonpolar organic chemicals in water[27,29] and to complex petroleum substances.[30,31] Furthermore, TLM-derived critical target lipid body burdens (CTLBBs) provide a basis for characterizing the range of sensitivities among aquatic organisms exposed to polar and nonpolar organics. The TLM, therefore, has potential to characterize relative sensitivities of aquatic organisms exposed to organic substances, including NAs, in OSPW.

Passive samplers have been commonly applied to measure freely dissolved concentrations of individual organic chemicals,[32] including recent applications to OSPW[33−35] and NAs.[36] Solid phase microextraction (SPME) has been adapted to measure the bioavailability of aqueous exposures petroleum hydrocarbon mixtures.[25,37−39] When relatively small volumes of SPME polymers are used in aqueous exposures, the freely dissolved oil concentrations in solution that dictate toxicity are not depleted, and the chromatographic area under the curve for all constituents absorbed onto the SPME fiber can be used as a surrogate measure for quantifying the bioavailability of petroleum contaminated samples. This is referred to as a biomimetic extraction (BE).[25] Prior work has established the technical basis for using BE as an empirical surrogate measure of bioavailable hydrocarbons.[40] Results of that study demonstrated that critical effect concentrations (e.g., LC50 and EC25) based on BE are equivalent to CTLBBs based on the TLM. Thus, a logical next step is to apply this approach to quantify the bioavailability of organic mixtures present in OSPW.

The first objective of the study was to use the TLM to characterize the relative sensitivity and general modes of toxic action of organic acids. The resulting species sensitivity distribution (SSD) was then compared to the SSD observed for aquatic organisms exposed to other polar and nonpolar organic chemicals. The second objective was to validate the BE method for application to organic acids commonly found in OSPW. In the present study, this was done by demonstrating that partitioning of test chemicals to polydimethylsiloxane (PDMS) under acidic conditions was consistent with partition coefficients measured for liposomes and fish bioconcentration factors measured under neutral (e.g., unacidified) conditions.[33,35] The guiding hypothesis is that BE measurements of acidified samples of organic acids is predictive of observed toxicity. BE measurements were used to evaluate concentration-responses for a variety of test substances, including single chemicals and extracts of OSPW and fractions thereof, to aquatic species. These data were used to develop a SSD based on BE, which was then compared with SSDs developed for BE derived previously using petroleum substances and to CTLBBs predicted by the TLM. The final objective was to integrate study findings into recommendations on developing BE-based water quality benchmarks for OSPW.

MATERIALS AND METHODS

The present study included three phases, leveraging distinct data sets to demonstrate the proof of concept on which the BE-based approach to developing water quality benchmarks is based (Table 1). The first phase involved application of the TLM to predict acute toxic potencies of individual chemicals. The goal of this work was to demonstrate that the sensitivities of aquatic species to organic acids were similar to those observed for nonpolar organics. The modeling analysis also evaluated the role of pH and ionization of organic acids on toxic potency.

The second phase developed a novel set of toxicity data with fish embryo tests for *Danio rerio* as well as tests with *Ceriodaphnia dubia*, and BE measurements were collected as the primary exposure metric. The main goal of this phase was to demonstrate that BE provided an acceptable metric of exposure for a series of test chemicals that spanned a range of numbers of carbon atoms and structures for the types of organics in OSPW. This initial validation work included measuring toxicity and BE for individual NAs, defined mixtures of those chemicals, and limited testing with acid extractable organics derived from OSPW.

The third phase was performed to leverage existing toxicity data reported in the literature for organic material extracted from OSPW. In this case, measurements of BE were performed on test substances at similar concentrations to those used in these studies (Table 1), which were then extrapolated to the actual toxicity data to derive BE-based critical effect concentrations. As a result, the existing training data set was expanded to include more species and test substances.

Phase I: Modeling Analysis of Literature Data. Toxicity data for organic acids were collected from the literature. Key search terms used in Google Scholar included: naphthenic acid, organic acid, and toxicity. Once key articles were identified, the Forward Search function of Google Scholar was employed to identify similar articles. The identified literature included primary sources and existing compilations. These data were used to test agreement with the TLM framework and are supplied in the Supporting Information (Table S1).

In addition to data for LC50 or EC50 and pH of exposures, select physicochemical properties were compiled: molecular weight (MW), log K_{OW} of neutral forms of compounds, and pK_a values for ionizable functional groups. EPISuite[41] was used to predict log K_{OW}, Absolv[42] was used to predict coefficients for the polyparameter TLM (ppTLM), and SPARC[43] was used to estimate pK_a (Table S1). Toxicity data were all converted to molar units.

Some effects data were based on nominal exposure concentrations. Experimental evidence from the current study and examples from the literature suggest that concentrations of

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Table 1. Outline of Research Elements in the Present Study

phase	description	objectives	key outcome	test substances	test species
1	target lipid model (TLM) analysis of literature toxicity data.	apply TLM on organic acids (Figure S2) evaluate role of pH (Figures S3 and S4)	TLM is fit for purpose of evaluating bioavailability of organic constituents in OSPW and supports technical basis of BE	single chemical phenols and carboxylic acids (Table S3)	fish and invertebrates (Table S4)
2	new experimental data on organic acids with BE measurements	develop validation data for BE using single chemicals with known properties, and in defined and extracted mixtures (Figures 3 and 4) develop acute to chronic ratios for organic acids (Figure S5)	demonstrated that BE method is applicable to single chemicals and in simple mixtures. Acute to chronic ratios consistent with those for other organics.	single naphthenic acids (Table S2), limited testing with OSPW extracts	fish embryo testing with *D. rerio* and reproduction testing with *C. dubia* (Tables2 and S2)
3	recent experimental data from literature on extracts of organic material from OSPW	develop BE data on substances used in other studies to expand data set measurements were collected on selected concentrations as a basis for extrapolation to the original toxicity test data (Table S1 and 2 and Figure 1)	expanded BE-based SSD to additional species and provided broader set of test substances	extracts from OSPW ponds using various extraction techniques (Table S3)	additional species include fish, invertebrates, and microbial end points (Table2)

organic acids are typically stable during acute toxicity tests (2−4 d), with measured concentrations generally within 80−120% of nominal. Therefore, variability introduced by the inclusion of toxicity results based on nominal values was likely small.

Relative proportions of neutral (protonated) and ionized forms of compounds were characterized by use of the Henderson−Hasselbalch equation (eq 1) to calculate the fraction of the neutral form of the chemical based on the pK_a's of substances and toxicity test pH.

$$\mathrm{pH} = \mathrm{p}K_a + \log(\mathrm{A}^-/\mathrm{HA}) \quad (1)$$

where A^- represents the ionic form of the chemical, and HA is the protonated, neutral form of the chemical. This provided a basis for evaluating the effects of chemical speciation (e.g., ionization) on observed toxic potencies, since pK_a values spanned nearly 8 log units, while under typical tests conditions were at pH of 7−8, resulting in wide variation in ionization across the larger data set.

The TLM is a model that predicts critical body burden, which relates toxicity to accumulation in target tissues (e.g., target lipids primarily in membranes) relative to a critical effects threshold (eq 2).

$$\log \mathrm{LC50} = -\log K_{\mathrm{LW}} + \log \mathrm{CTLBB} \quad (2)$$

The CTLBB (μmol/g lipid) is the concentration of chemicals in target lipids, which represents sites of action for these types of organic chemicals, and K_{LW} is the target lipid−water partition coefficient. The CTLBB represents tolerances and thresholds for effect of chemicals that are a function of test species and end point.

The K_{LW} was estimated using the poly parameter linear solvation-energy relationship (LSER) of lipid−water (e.g., LSER-based TLM).[28] The general form of the poly parameter LSER model is given (eq 3).

$$\log K = eE + sS + aA + bB + vV + c \quad (3)$$

where lowercase parameters (e, s, a, b, and v) correspond to the solvent system (e.g., target lipid−water), and the uppercase parameters (E, S, A, B, and V) are chemical interaction terms for solutes. The parameter E is excess molar refractivity, S is polarizability, A is ability to donate a hydrogen bond, B is ability to accept a hydrogen bond, and V is molar volume. The term c is a fitting constant and accounts for unit conversions between different phases and uncertainties in the partitioning process that are not explicitly described by the model in eq 3. This general LSER modeling approach is widely applied to partitioning data and is described in more detail elsewhere.[44−46]

The LSER approach was used to predict K_{LW} (eq 4) for the neutral form of the chemical.[28]

$$\log K_{\mathrm{LW}} = 0.51E + 0.71S + 0.92A - 4.40B + 3.14V - 0.44 \quad (4)$$

Ionized forms of chemicals are also expected to bioaccumulate and contribute to toxicity.[47−53] The lipid−water partition coefficient for the ionizable form of the chemical (K_{LW_ion}) was assumed to be proportional to the neutral form (K_{LW}). Therefore, an existing model framework from the literature was used to estimate K_{LW_ion}[49−51] (eq 5), where the K_{LW_ion} is calculated as a constant fraction (ΔMW) of K_{LW}, where ΔMW is the ratio between the ionic and neutral form partition coefficients.

$$\log K_{\mathrm{LW\_ion}} = \Delta\mathrm{MW}\log K_{\mathrm{LW}} \quad (5)$$

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The reduced affinity of the ionic species for biological tissues relative to the neutral form is likely due to the more polar nature of the ionized chemicals.[54] However, the partitioning of the ionic form is not negligible and is often greater than can be theoretically explained by the protonated form.[53] While the mechanism by which the ionic forms are toxic is unclear, the processes, which govern the partition coefficient (K_{LW_ion}) are complex[55] and includes passive diffusion[53] and active transport[56,57] mechanisms.

The TLM (eq 2) was modified to account for ionization using a composite lipid water partition coefficient (D_{LW}) for the fraction ionized (f_{ion}; eq 1) and the two partition coefficients for the neutral (eq 4) and ionic (eq 5) constituents.

$$\log D_{LW} = \log((1 - f_{ion})K_{LW} + f_{ion}K_{LW\_ion}) \quad (6)$$

Thus, the TLM was fit to experimental toxicity data (LC50) by optimizing the logCTLBB (eq 7).

$$\log \mathrm{LC50} = -\log D_{LW} + \log \mathrm{CTLBB} \quad (7)$$

The ratio of partition coefficients for the ionic and neutral form (eq 5) was estimated by fitting to the compiled data set using Solver in Excel by minimizing the error in the model fit (eq 7). In this study it was initially assumed that organic acids acted as narcotic chemicals, which is consistent with other experimental and modeling studies.[12] The validity of this assumption was evaluated by comparing CTLBBs derived from empirical effects data found in the literature to the species sensitivity distribution found in the TLM.[27]

Phase 2: Development of Paired Toxicity and BE Data. The toxicity testing and conventional chemical analysis protocols are summarized in this section. More detailed methods are given in the Supporting Information.

Toxicity Tests. Toxicity tests were conducted for individual compounds that spanned a range of chemical properties, defined mixtures of those compounds, a commercially available mixture of NAs (Merichem, Houston, TX, U.S.A.),[58] and a mixture of acid extractable organics isolated from OSPW provided by Environment and Climate Change Canada (ECCC, Burlington, ON, Canada;[59] Table S2). All chemicals obtained from commercial sources were ≥98% purity. Toxicity testing was conducted using zebrafish (*Danio rerio*) embryos and the water flea (*Ceriodaphnia dubia*).

Zebrafish (D. rerio) Embryo Toxicity Tests. Toxicity to zebrafish embryos was evaluated according to OECD protocol 236.[60] Embryos were exposed for 4 days at 28 ± 1 °C. Microscopic observations were performed at 24 ± 1 h intervals. End points recorded included number of coagulated eggs, lack of somite formation, and tail-bud detachment from yolk sac. Following the first 24 h observation, the remaining end point observed was lack of heartbeat. Mortality was determined by coagulation or absence of heartbeat.

Ceriodaphnia dubia 3-Brood Reproduction Assay. Testing with *C. dubia* followed the ECCC test guideline,[61] where 6−7 day exposures were conducted at 25 ± 2 °C with a photoperiod of 16:8 h light/dark. Exposure waters were renewed daily. Observations for immobilization were performed and recorded at approximately 24 h intervals following test initiation for up to 7 d. Following test completion, the total number of living offspring produced per living female at the end of the test was assessed.

Preparation of Toxicity Test Solutions. A concentrated stock solution of each model compound was prepared 2 days before initiating tests and a dilution of each treatment was made on the day of initiation. The stock solutions of individual chemicals and defined mixtures were maintained at pH to 7.3 (± 0.2) and adjusted as needed, and exposure solutions were mixed 24 h prior to test initiation at test temperature with Teflon-coated stir bars.

Exposure solutions for some sparingly soluble chemicals, including tridecanoic and cholanic acids, were developed by use of a glass generator column, a tube plugged with glass wool and packed with the powdered test material. Exposures were developed by pumping the exposure solution through the columns at a flow of approximately 0.8 mL/min. Eluates from columns (test solutions) were collected in 1 L glass bottles. These compounds were tested individually at a single concentration (the solubility limit for each compound).

Analytical Confirmation of Test Substance Exposure Concentrations. Conventional Analysis. Water samples from the control and each exposure concentration were extracted via solid phase extraction (SPE) using 1 mL (100 mg) octadecyl (C_{18}) J.T. Baker Bakerbond cartridges (VWR). Diluted, acidified samples were also spiked with 50−150 μL of 200−2000 μg/mL internal standards. Cartridges were eluted with formic acid in methanol or methylene chloride. Extracts were then exchanged into hexane. Sample extracts were quantified by use of gas chromatography, with flame ionization detection (GC-FID).

Biomimetic Extraction (BE) by Solid Phase Microextraction. Samples for analysis by BE were acidified to pH ∼2.4 in 20 mL vials with no headspace and sealed with Teflon-faced septum caps. The BE analysis was performed automatically by introducing an SPME fiber coated with 30 μm polydimethylsiloxane (PDMS) into the aqueous sample for 100 min at 30 °C with rapid agitation (250 rpm) using large water-to-PDMS ratios such that analyte was not depleted[25] and generally sufficient to reach equilibrium.[40] Equilibrated fibers were placed into the GC-FID. The FID response was normalized against 2,3-dimethylnaphthalene derived from liquid solvent injection of a series of hydrocarbon standards. BE results were normalized to the PDMS volume (0.132 μL) on the fiber and reported as millimoles (mmol)/liter (L) PDMS.[25] BE measurements on the samples used in the present study that were not acidified resulted in very low accumulation.

Phase 3: Toxicity Data from Literature on Extracts from OSPW. Toxicity data derived from exposures to extracts from OSPW collected from sources in the literature were used to supplement data developed in the present study (Table 2 and S2−S4).

The first study included exposures to three acid extracts of organic materials from OSPWs, which were considered to be comprised of mainly NA molecules.[59] Toxicity data included exposures to *Hyalella azteca* (amphipod), *Vibrio fischeri* (bacteria), *Lampsilis cardium* (freshwater mussel),[62] and embryos of *Pimephales promelas* (fathead minnow).[59] Extracts were prepared from OSPW collected from holding ponds from various surface mining operators in the Canadian Oil Sands region. Toxicity testing on fathead minnow, daphnids, and algae was performed using Merichem NAs.[58]

A third source of data[3] was a study in which toxicity testing was conducted with extracts (including ionizable, polar, and nonpolar constituents) of OSPW that had been collected from Base-Mine Lake, an end-pit lake at the Syncrude operation. Toxicity data for these extracts were obtained from exposures of embryos of *P. promelas*.[3]

Measurements of BE were performed on a series of the same extracts used in testing during these studies at concentrations

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Table 2. Critical Biomimetic Extraction (BE) Concentrations for Acute and Chronic, Acute to Chronic Ratios, and Standard Errors[a]

species	acute log BE (mM)	SE	end point	chronic log BE (mM)	SE	end point	acute to chronic ratio
D. rerio	1.66	0.23	4 d embryo survival, LC50	1.36	0.5	4 d embryo deformity, EC25	2.00
P. promelas	1.29	0.2	4 d embryo survival, LC50	0.99	0.24	4 d embryo sublethal (deformity, heart rate), EC25	2.00
D. magna	1.62	0.33	2 d survival, LC50				
Microtox	2.01	0.15	15 min IC50				
C. dubia	1.77	0.18	4 d survival, LC50	1.07	0.15	7 d reproduction, EC25	5.01
H. azteca	1.49	0.16	7 d survival, LC50	1.05	0.16	28 d survival, LC50	2.75
L. cardium	2.25	0.37	2 d viability, LC50				
P. subcapitata	1.92	0.24	3 d growth rate, EC50	1.57	0.27	3 d growth rate, EC25	2.24

[a]SE is the standard error.

that were similar to those used in the toxicity tests. These data were then used to extrapolate estimates of BE to actual test concentrations. This was done by calculating the average response (BE/concentration) for each substance (Figure S1). The BE/concentration ratio (Table S1) was calculated for each substance and applied to the concentration−response data found in the primary literature. All of the BE-response data used in the present study are provided in Table S4.

Statistical Analyses. SSDs, which are ranked compilations of critical effect concentrations (e.g., LC50 and EC25), are commonly used as a tool for developing water quality benchmarks.[63−65] The acute, sublethal, and chronic end points evaluated in the SSD in this study are shown in Table 2. Logistic regressions were performed using *dose.p* in the MASS package in R.[66] This included determination of EC25s for chronic and sublethal data, LC50s for acute mortality data, as well as 95% confidence intervals of the logistic regressions.

RESULTS

Phase 1: Modeling Analysis of Literature Data. The data set compiled from the literature contains 678 individual entries for approximately 300 individual organic chemicals for several fishes, invertebrates, algae, and microbes (Table S1). These constituents spanned log D_{LW} from approximately −1 to +7 and included NAs (e.g., fatty acids and cyclic carboxylates, n = 148), as well as substituted benzoic acids (n = 55) and phenols (n = 475). Carboxylate and benzoate functional groups have pK_a values near 4.8. The pK_a values for substituted phenols varied from 3 to 10 but were generally between 6 and 9.

The frequency distribution of reported LC50s as a function with log D_{LW} was linear (eq 7, Figure S2). The root-mean-square error was 0.72, which is somewhat higher than the performance of the TLM for other polar and nonpolar organics, reflecting the challenging nature of ionic chemicals.[28] The fitted ΔMW (eq 5) was 0.045 (0.098−0.024), which is within the reported range (0.1−0.01) for ionizable organics.[54,67] Also, some chemicals exhibited *di minimiz* toxic potencies (>symbols) at DLW > 5, or at pH 9. This is consistent with prior TLM applications to nonpolar organics,[68] indicating little bioavailability for those chemicals in that exposure system.

For some classes of chemicals, such as carboxylates, the ionized fraction likely dominates at the usual pH values of test solutions (pH 7−8). The role of ionization on model performance was evaluated by comparing predictions from the model to the fraction of the chemical that is computed to be in the ionizable form (lines vs symbols, Figure S3) based on test pH and substance pK_a (eq 1). For example, replotting Figure S2 using LC50s that have been corrected for the fraction of nonionized material, and with partition coefficient for the neutral form (log K_{TL}, eq 3), resulted in systematically poorer performance (Figure S3).

There were no discernible trends in model residuals vs ionization state among classes of chemicals studied based on data from the literature (Figure S4). Some effects of pH on toxicity have been observed.[69,70] However, these effects were relatively minor and were not correlated with the magnitude of the shift in fractions of ionized chemicals. Therefore, it was concluded that the effects of pH on toxic potencies of organic acids can be important, but are secondary to other chemical-physical characteristics. While log D_{LW} appears to be a reasonably good descriptor of observed toxicity, normalization to neutral forms of chemicals does not improve relationships, a result that is consistent with results of other modeling studies of NAs.[71]

The data set used in the present study provided CTLBBs for 14 species including algae, bacteria, fish, snails, and other invertebrates (Table S5). The magnitude and distribution for this subset of chemicals and species are similar to those used to derive the TLM-derived SSD for other polar organics (Figure 1). Success of the TLM in describing toxicities of organic acids and similarities of resulting SSDs suggests that the classes of chemicals studied exhibit similar baseline modes of action as do other polar and nonpolar organics. This is consistent with previously published results[12] and supports the application of the BE method as a unifying exposure metric, since these classes of chemicals are expected to have similar and additive modes of toxic action.

Phases 2 and 3: Biomimetic Extraction as Exposure Metric. The BE method is based on partitioning of organics to PDMS under acidic conditions (K_{PDMS}). It was assumed that this is representative of partitioning of ionizable organic acids to biological tissues. This is due to the relatively small effect of ionization of organic acids (ΔMW range 0.1−0.01, eq 3) on observed toxicity, especially considering the degree of ionization by comparing chemical pK_a to test pH, which are often more than three log units different. During the present study, this assumption was confirmed by comparing measured PDMS-water partition coefficients in acidified solutions (the present study) to partition measurements for organic acids to liposomes and measured bioconcentration factors (BCF), for OSPW constituents[35] and model compounds.[54]

All measurements increased as a function of numbers of carbons. Measured log BCFs for constituents of OSPW ranged from 1.5 to 3.2 among numbers of carbon from 11 to 19 (Figure 2).

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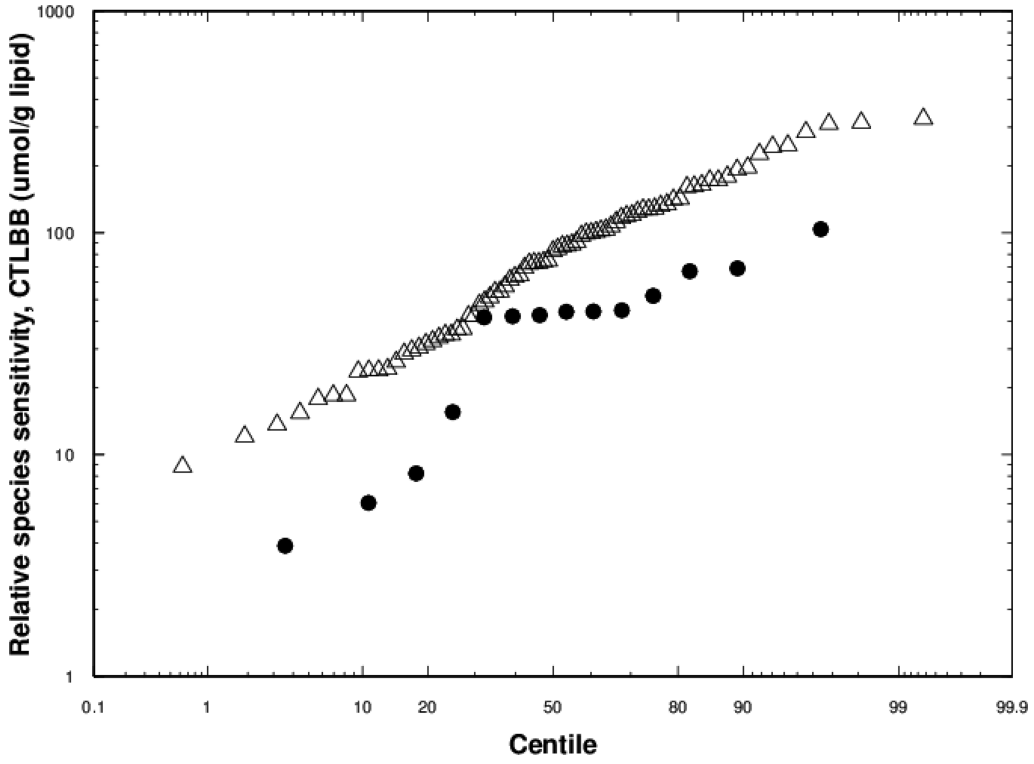

Figure 1. Comparing species sensitivity distribution from target lipid model (TLM)-derived critical target lipid body burdens (CTLBB in eq 7) for polar and ionizable organics from a recent update to the TLM (triangles)[73] to CTLBBs derived for ionizable organics compiled during the present study (circles).

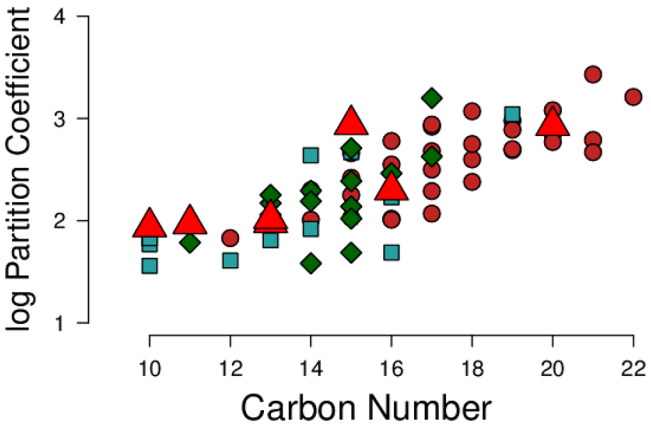

Figure 2. Comparing polydimethylsiloxane (PDMS)-water partition coefficients measured at low pH in the present study (▲, see Materials and Methods) to measured bioconcentration factors (♦), liposome partition coefficients (●) measured at pH 7.4 for constituents in OSPW,[35] and measured liposome partition coefficients for model compounds (■),[54] against total carbons in the molecule. Data in this figure include model compounds and those from OSPW,[33] which have one carboxylate group with 1−3 double bond equivalents (e.g., rings).

The measured log liposome partition coefficients were consistent among studies and ranged from 1.8 to 3.4 among numbers of carbon from 10 to 22 (Figure 2). Acidified log K_{PDMS} exhibited a similar range of 1.9−2.9 among numbers of carbons from 10 to 20. Similarities between K_{PDMS} and the other biology-based partitioning metrics validates use of the BE method as a surrogate measurement of bioavailability. This is supported by the observed correlation of fiber-water partition coefficients with BCFs for anionic surfactants, including C10−18 carboxylates.[72] Direct comparisons within this data set are not possible because there were different chemicals among data sets. However, the observed correlation demonstrates that K_{PMDS} on acidified samples approximates BCF values measured for fish and liposome partition coefficients and empirical BCF data establishes proof of concept that acidified BE measurements are a reasonable surrogate for quantifying bioavailability.

The composite data set used in the present study includes new experimental data and data from the literature (Tables 1, 2, and S3), such as acid extractable organics from OSPW, a commercial mixture of NAs (Merichem), and chemicals tested individually and in defined mixtures. This data set included eight species for which data on acute lethality were available and four species for which chronic, or sublethal toxicity data, were available.

Data for acute toxicity included fish embryos (*D. rerio* and *P. promelas*), invertebrates (*C. dubia*, *D. magna*, *H. azteca*, and *L. cardium*), bacteria (*V. fischeri*) and algae (*P. subcapitata*; Figure 3). Measured acute end points included lethality or reduced growth rate (algae), inhibition of luminescence (bacteria), and valve closure (mussel larvae). Sublethal effects included observations of embryo deformities and heart rates for *P. promelas*. Chronic effects included cumulative production of neonates over three broods of *C. dubia*. In some cases the effects data were developed in separate laboratories using different test substances, which is notable since BE measurements provided a consistent exposure metric across the data set, especially for end points that included various types of test substances.

To characterize relative sensitivity and variability among end points, critical BE concentrations (LC50s based on the BE) were derived using logistic regressions (Table 2). The log-acute critical fiber concentrations (mM) ranged from 1.43 to 2.17, with a median of 1.79 and a standard deviation of 0.26 (Figure 4). Mean relative standard error of individual log-transformed end points was 0.23 with a range of 0.16−0.37. The TLM-derived

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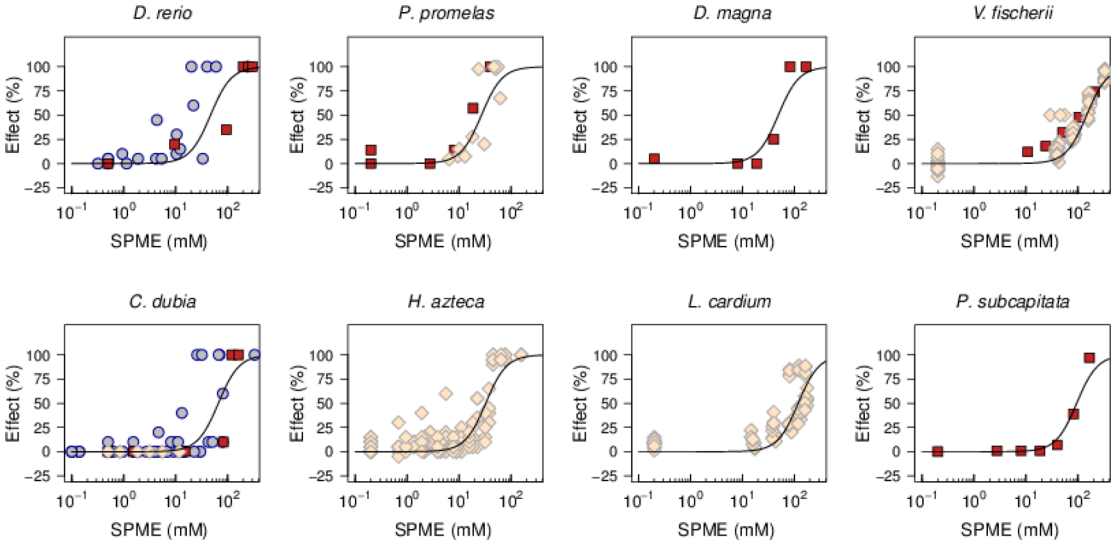

Figure 3. Acute concentration−response data using biomimetic extraction (BE) as the exposure metric for single chemicals (●), defined mixtures of single chemicals and commercial mixtures (■), and acid extractable organics from oil sands process-affected water (OSPW; ♦). Curves are logistic regressions of effects data.

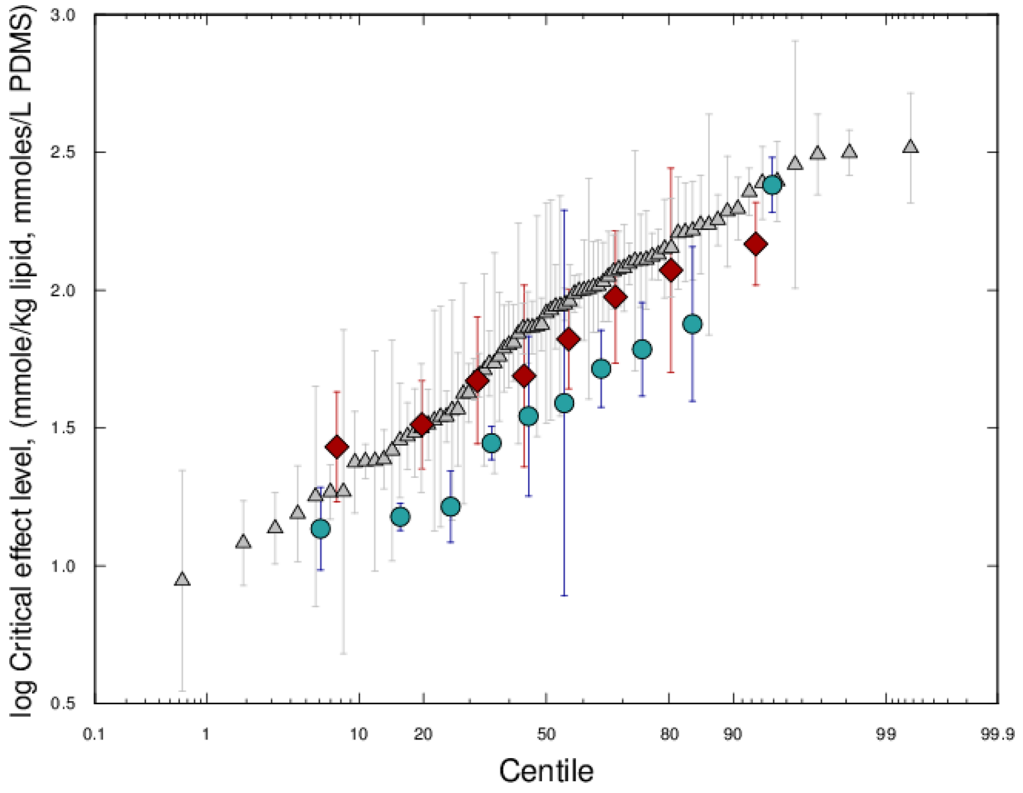

Figure 4. Comparing critical fiber concentrations and standard errors (mmol/L polydimethylsiloxane (PDMS)) derived for hydrocarbons (●),[40] and organic acids (♦, present work), and target lipid model (TLM)-derived tissue-based effect concentrations (mmol/kg lipid, ▲).[73]

distribution of log CTLBBs had a mean of 1.85 and a standard deviation of 0.36, with a relative standard error of the individual log CTLBBs of approximately 0.40 for each specific end point.[73] In addition, critical BE concentrations observed during the present study were similar in magnitude and distribution to critical BE concentrations determined for hydrocarbon mixtures derived from aquatic toxicity testing of petroleum substances (circles, Figure 3). Collectively, these observations (Figures 2−5) support the use of BE-SPME as a tool to predict toxicity during exposures to complex mixtures, including organic constituents of OSPW.

Data on chronic effects exhibited similar concentration−response patterns, indicating that BE seems to provide consistent exposure metrics among types of substances. The log chronic critical threshold concentrations (e.g., 25% response, Table 2 and Figure 5) ranged from 0.99 to 1.57 for four distinct end points: sublethal deformity, heartbeat, reduced growth, and reproduction. This limited data set of chronic end points provides some indication of concentrations associated with thresholds of chronic effects.

Acute to Chronic Ratios. The data used in the modeling analysis (Phase 1) were mainly acute toxicity data. Derivation of

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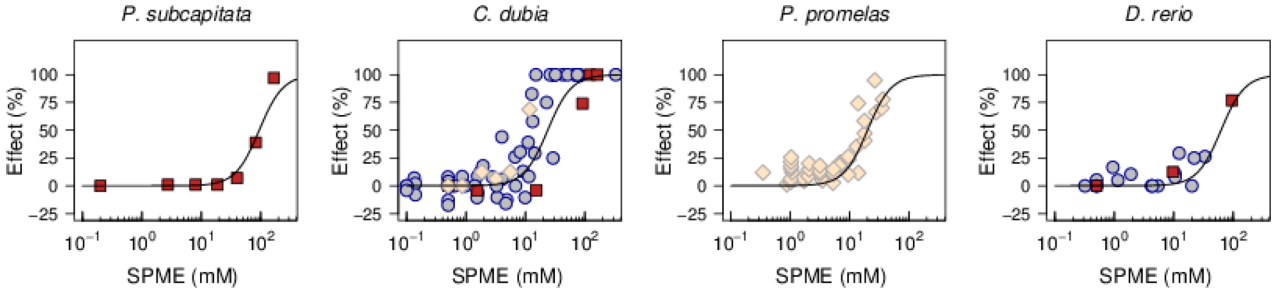

Figure 5. Concentration−response relationships for growth of algae (same data in Figure 3), reproduction of *C. dubia*, and sublethal effects on embryos of *P. promelas* and *D. rerio* with BE as the exposure metric for single chemicals (●), defined mixtures of single chemicals and commercial mixtures (■), and acid extractable organics from oil sands process-affected water (OSPW; ♦). Curves are logistic regressions of effects data.

water quality benchmarks often include extrapolation of acute thresholds to chronic thresholds using acute to chronic ratios (ACRs).[63] The experimental data developed during the present study resulted in ACRs that ranged from 1.5 to 10 (Figure S5). The ACRs based on conventional exposure metrics (e.g., mg/L of organic chemical in water) are comparable to ACRs derived using BE as the exposure metric.

ACRs computed for the acute and chronic critical BE concentrations ranged from 2.0 to 5.0, which is in the range of ACRs for other nonpolar and polar chemicals acting through acute narcosis (Figure S5, Table 2). The BE-based ACRs for tests with single chemicals ranged from 1 to 31.5 (Table S2), with an average of 7.5. This limited data set continues to support the assumed similar toxic modes of action for acute as well as subacute and chronic effects, as well as the assumption of additivity.

DISCUSSION

The major outcome of this study was development of passive sampler methods to support derivation of water quality benchmarks for organic constituents in OSPW. Thresholds based on BE were developed for several invertebrates and fishes exposed to organics extracted from OSPW. These data form the basis of an initial SSD that could potentially support the derivation of water quality benchmarks by characterizing the range of acute and chronic thresholds of organic substances related to OSPW. Additional data on sensitive species, and importantly, across a range of OSPW from various operations and ages are needed to improve the technical basis for applying this approach.

Mechanisms that control partitioning of nonpolar organic chemicals to lipid and PDMS are similar[40] and provide a theoretical basis for application of BE as a surrogate measurement of bioavailability for exposures to complex organic mixtures. Mechanisms that control toxicity, and implied bioaccumulation, of ionizable organics are not well-resolved and likely involve some combination of passive and active uptake.[57] The modeling employed in the present study, and other observations in the literature, indicate that the K_{LW} values for the ionic forms covary with those of the neutral forms, and a composite D_{LW} was able to describe observed structure−activity relationships.[49,67,74] The modeled D_{LW} was found to be analogous to K_{PDMS} measured by use of the BE method (Figure 2).

Measurements of BE for OSPW-derived samples were performed on acidified samples, ensuring that the carboxylic acid groups were protonated to promote absorption on to the SPME fiber. The present study demonstrates that acidified BE measurements provide a reasonable basis for evaluating the toxicity of both neutral and ionic forms of organic mixtures in OSPW.

The BE method was evaluated by use of model compounds and extracts of organic material derived from OSPW, and provides a flexible exposure metric that accommodates variable compositions inherent in OSPW.[22] The initial validation presented here confirms the technical basis for using BE as a basis for the derivation of water quality benchmarks for the organic constituents within OSPW. Additional validation could include a broader range of acute and chronic data and test substances to ensure the derivation of comprehensive BE-based water quality benchmarks.

The BE method presented here could serve as a rapid and convenient analytical screening tool for estimating the toxicity of raw and treated OSPWs, relative to performing whole effluent testing with various species, which can require several days or weeks to complete. Therefore, BE measurements have the potential to streamline the water quality assessment of treated OSPW effluents by providing timely and cost-effective data on the predicted toxicity of the organics in a given effluent. Furthermore, the BE method can be applied to treated OSPW at ambient pH, and under acidified conditions, to characterize relative contributions from hydrocarbons (e.g., bitumen, PAH, and residual solvent) and organic acids, respectively.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b00614.

Additional tables of literature and experimental data (XLSX)

Additional information related to toxicity test and exposure methods including conventional analytical methods and additional figures supporting data extrapolation and modeling analysis (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Clemente, J. S.; Fedorak, P. M. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere* **2005**, *60* (5), 585−600.

(2) Rowland, S. J.; Scarlett, A. G.; Jones, D.; West, C. E.; Frank, R. A. Diamonds in the rough: identification of individual naphthenic acids in oil sands process water. *Environ. Sci. Technol.* **2011**, *45* (7), 3154−3159.

(3) Morandi, G. D.; Wiseman, S. B.; Pereira, A.; Mankidy, R.; Gault, I. G.; Martin, J. W.; Giesy, J. P. Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. *Environ. Sci. Technol.* **2015**, *49* (20), 12395−12404.

(4) Headley, J. V.; Barrow, M. P.; Peru, K. M.; Fahlman, B.; Frank, R.; Bickerton, G.; McMaster, M.; Parrott, J.; Hewitt, L. Preliminary fingerprinting of Athabasca oil sands polar organics in environmental samples using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Rapid Commun. Mass Spectrom.* **2011**, *25* (13), 1899−1909.

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

(6) Barrow, M. P.; Witt, M.; Headley, J. V.; Peru, K. M. Athabasca oil sands process water: Characterization by atmospheric pressure photoionization and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* **2010**, *82* (9), 3727−3735.

(7) Grewer, D. M.; Young, R. F.; Whittal, R. M.; Fedorak, P. M. Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? *Sci. Total Environ.* **2010**, *408* (23), 5997−6010.

(8) Pereira, A. S.; Bhattacharjee, S.; Martin, J. W. Characterization of oil sands process-affected waters by liquid chromatography orbitrap mass spectrometry. *Environ. Sci. Technol.* **2013**, *47* (10), 5504−5513.

(9) Headley, J. V.; Peru, K. M.; Barrow, M. P. Advances in mass spectrometric characterization of naphthenic acids fraction compounds in oil sands environmental samples and crude oil—a review. *Mass Spectrom. Rev.* **2016**, *35* (2), 311−328.

(10) Hughes, S. A.; Mahaffey, A.; Shore, B.; Baker, J.; Kilgour, B.; Brown, C.; Peru, K. M.; Headley, J. V.; Bailey, H. C. Using ultrahigh-resolution mass spectrometry and toxicity identification techniques to characterize the toxicity of oil sands process-affected water: The case for classical naphthenic acids. *Environ. Toxicol. Chem.* **2017**, *36* (11), 3148−3157.

(11) Yue, S.; Ramsay, B. A.; Wang, J.; Ramsay, J. Toxicity and composition profiles of solid phase extracts of oil sands process-affected water. *Sci. Total Environ.* **2015**, *538*, 573−582.

(12) Frank, R. A.; Kavanagh, R.; Burnison, B. K.; Arsenault, G.; Headley, J. V.; Peru, K. M.; Van Der Kraak, G.; Solomon, K. R. Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. *Chemosphere* **2008**, *72* (9), 1309−1314.

(13) Frank, R. A.; Fischer, K.; Kavanagh, R.; Burnison, B. K.; Arsenault, G.; Headley, J. V.; Peru, K. M.; Kraak, G. V. D.; Solomon, K. R. Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. *Environ. Sci. Technol.* **2009**, *43* (2), 266−271.

(14) Headley, J. V.; McMartin, D. W. A review of the occurrence and fate of naphthenic acids in aquatic environments. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2004**, *39* (8), 1989−2010.

(15) Melvin, S. D.; Lanctôt, C. M.; Craig, P. M.; Moon, T. W.; Peru, K. M.; Headley, J. V.; Trudeau, V. L. Effects of naphthenic acid exposure on development and liver metabolic processes in anuran tadpoles. *Environ. Pollut.* **2013**, *177*, 22−27.

(16) Li, C.; Singh, A.; Klamerth, N.; McPhedran, K.; Chelme-Ayala, P.; Belosevic, M.; Gamal El-Din, M. Synthesis of Toxicological Behavior of Oil Sands Process-Affected Water Constituents. *OSRIN Report No. TR-50*; Oil Sands Research and Information Network, University of Alberta: Edmonton, Alberta, 2014.

(17) Martin, J. W. The Challenge: Safe release and reintegration of oil sands process-affected water. *Environ. Toxicol. Chem.* **2015**, *34* (12), 2682−2682.

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

(19) Giesy, J. P.; Anderson, J. C.; Wiseman, S. B. Alberta oil sands development. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (3), 951−952.

(20) Siwik, P. L.; Van Meer, T.; MacKinnon, M. D.; Paszkowski, C. A. Growth of fathead minnows in oilsand-processed wastewater in laboratory and field. *Environ. Toxicol. Chem.* **2000**, *19* (7), 1837−1845.

(21) Anderson, J.; Wiseman, S. B.; Moustafa, A.; Gamal El-Din, M.; Liber, K.; Giesy, J. P. Effects of exposure to oil sands process-affected water from experimental reclamation ponds on Chironomus dilutus. *Water Res.* **2012**, *46* (6), 1662−1672.

(22) Frank, R. A.; Milestone, C. B.; Rowland, S. J.; Headley, J. V.; Kavanagh, R. J.; Lengger, S. K.; Scarlett, A. G.; West, C. E.; Peru, K. M.; Hewitt, L. M. Assessing spatial and temporal variability of acid-extractable organics in oil sands process-affected waters. *Chemosphere* **2016**, *160*, 303−313.

(23) Douben, P. E. *PAHs: an ecotoxicological perspective*; John Wiley & Sons: New York, 2003.

(24) Redman, A. D.; Parkerton, T. F. Guidance for improving comparability and relevance of oil toxicity tests. *Mar. Pollut. Bull.* **2015**, *98* (1), 156−170.

(25) Letinski, D.; Parkerton, T.; Redman, A.; Manning, R.; Bragin, G.; Febbo, E.; Palandro, D.; Nedwed, T. Use of passive samplers for improving oil toxicity and spill effects assessment. *Mar. Pollut. Bull.* **2014**, *86* (1), 274−282.

(26) Di Toro, D. M.; McGrath, J. A.; Hansen, D. J. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. *Environ. Toxicol. Chem.* **2000**, *19* (8), 1951−1970.

(27) McGrath, J. A.; Toro, D. M. D. Validation of the target lipid model for toxicity assessment of residual petroleum constituents: monocyclic and polycyclic aromatic hydrocarbons. *Environ. Toxicol. Chem.* **2009**, *28* (6), 1130−1148.

(28) Kipka, U.; Di Toro, D. M. Technical basis for polar and nonpolar narcotic chemicals and polycyclic aromatic hydrocarbon criteria. III. A polyparameter model for target lipid partitioning. *Environ. Toxicol. Chem.* **2009**, *28* (7), 1429−1438.

(29) McGrath, J. A.; Parkerton, T. F.; Di Toro, D. M. Application of the narcosis target lipid model to algal toxicity and deriving predicted-no-effect concentrations. *Environ. Toxicol. Chem.* **2004**, *23* (10), 2503−2517.

(30) Redman, A. D.; Parkerton, T. F.; McGrath, J. A.; Di Toro, D. M. PETROTOX: An aquatic toxicity model for petroleum substances. *Environ. Toxicol. Chem.* **2012**, *31* (11), 2498−2506.

DOI: 10.1021/acs.est.8b00614
Environ. Sci. Technol. 2018, 52, 8039−8049

(31) Redman, A. D.; Parkerton, T. F.; Leon Paumen, M.; Butler, J. D.; Letinski, D. J.; den Haan, K. A re-evaluation of PETROTOX for predicting acute and chronic toxicity of petroleum substances. *Environ. Toxicol. Chem.* **2017**, *36* (8), 2245−2252.
(32) Mayer, P.; Parkerton, T. F.; Adams, R. G.; Cargill, J. G.; Gan, J.; Gouin, T.; Gschwend, P. M.; Hawthorne, S. B.; Helm, P.; Witt, G.; et al. Passive sampling methods for contaminated sediments: Scientific rationale supporting use of freely dissolved concentrations. *Integr. Environ. Assess. Manage.* **2014**, *10* (2), 197−209.
(33) Zhang, K.; Pereira, A. S.; Martin, J. W. Estimates of Octanol−Water Partitioning for Thousands of Dissolved Organic Species in Oil Sands Process-Affected Water. *Environ. Sci. Technol.* **2015**, *49* (14), 8907−8913.
(34) Morandi, G. D.; Zhang, K.; Wiseman, S. B.; Pereira, A. d. S.; Martin, J. W.; Giesy, J. P. Effect of Lipid Partitioning on Predictions of Acute Toxicity of Oil Sands Process Affected Water to Embryos of Fathead Minnow (Pimephales promelas). *Environ. Sci. Technol.* **2016**, *50* (16), 8858−8866.
(35) Zhang, K.; Wiseman, S.; Giesy, J. P.; Martin, J. W. Bioconcentration of Dissolved Organic Compounds from Oil Sands Process-Affected Water by Medaka (Oryzias latipes): Importance of Partitioning to Phospholipids. *Environ. Sci. Technol.* **2016**, *50* (12), 6574−6582.
(36) Harman, C.; Langford, K.; Sundt, R. C.; Brooks, S. Measurement of naphthenic acids in the receiving waters around an offshore oil platform by passive sampling. *Environ. Toxicol. Chem.* **2014**, *33* (9), 1946−1949.
(37) Redman, A. D.; Butler, J. D.; Letinski, D. J.; Parkerton, T. F. Investigating the role of dissolved and droplet oil in aquatic toxicity using dispersed and passive dosing systems. *Environ. Toxicol. Chem.* **2017**, *36* (4), 1020−1028.
(38) Parkerton, T.; Stone, M.; Letinski, D. Assessing the aquatic toxicity of complex hydrocarbon mixtures using solid phase micro-extraction. *Toxicol. Lett.* **2000**, *112-113*, 273−282.
(39) Redman, A. D.; Parkerton, T. F.; Letinski, D. J.; Manning, R. G.; Adams, J. E.; Hodson, P. V. Evaluating toxicity of heavy fuel oil fractions using complementary modeling and biomimetic extraction methods. *Environ. Toxicol. Chem.* **2014**, *33* (9), 2094−2104.
(40) Redman, A.; Butler, J.; Letinski, D.; Di Toro, D.; Leon Paumen, M.; Parkerton, T. Technical basis for using passive sampling as a biomimetic extraction procedure to assess bioavailability and predict toxicity of petroleum substances. *Chemosphere* **2018**, *199*, 585−594.
(41) EPA, U. *Estimation Programs Interface Suite for Microsoft Windows*. U.S. Environmental Protection Agency: Washington, DC, 2012.
(42) ACD/Labs, Absolv data sheet. http://www.acdlabs.com/products/percepta/predictors/absolv/.
(43) Hilal, S.; Karickhoff, S.; Carreira, L. *Verification and validation of the SPARC model*; U.S. Environmental Protection Agency: Washington, DC, 2003.
(44) Abraham, M. H. Application of solvation equations to chemical and biochemical processes. *Pure Appl. Chem.* **1993**, *65* (12), 2503−2512.
(45) Endo, S.; Brown, T. N.; Goss, K.-U. General model for estimating partition coefficients to organisms and their tissues using the biological compositions and polyparameter linear free energy relationships. *Environ. Sci. Technol.* **2013**, *47* (12), 6630−6639.
(46) Endo, S.; Goss, K.-U. Applications of polyparameter linear free energy relationships in environmental chemistry. *Environ. Sci. Technol.* **2014**, *48* (21), 12477−12491.
(47) Escher, B. I.; Schwarzenbach, R. P.; Westall, J. C. Evaluation of Liposome−Water Partitioning of Organic Acids and Bases. 2. Comparison of Experimental Determination Methods. *Environ. Sci. Technol.* **2000**, *34* (18), 3962−3968.
(48) Escher, B. I.; Schwarzenbach, R. P.; Westall, J. C. Evaluation of Liposome−Water Partitioning of Organic Acids and Bases. 1. Development of a Sorption Model. *Environ. Sci. Technol.* **2000**, *34* (18), 3954−3961.
(49) Escher, B. I.; Sigg, L. Chemical speciation of organics and of metals at biological interphases. *IUPAC Series on Analytical and Physical Chemistry of EnVironmental Systems* **2004**, *9*, 205−270.
(50) Escher, B.; Sigg, L.; Van Leeuwen, H.; Köster, W. *Physicochemical Kinetics and Transport at Biointerfaces*. John Wiley & Sons, Ltd.: Chichester, U.K., 2004.
(51) Escher, B.; Kwon, J.-H. Development of an in vitro system for modeling bioaccumulation of neutral, ionizable, and metabolically active organic pollutants in fish. *ALTEX: Alternatives to Animal Experimentation* **2007**, *24* (Supp), 81−82.
(52) Erickson, R. J.; McKim, J. M.; Lien, G. J.; Hoffman, A. D.; Batterman, S. L. Uptake and elimination of ionizable organic chemicals at fish gills: II. Observed and predicted effects of pH, alkalinity, and chemical properties. *Environ. Toxicol. Chem.* **2006**, *25* (6), 1522−1532.
(53) Erickson, R. J.; McKim, J. M.; Lien, G. J.; Hoffman, A. D.; Batterman, S. L. Uptake and elimination of ionizable organic chemicals at fish gills: I. Model formulation, parameterization, and behavior. *Environ. Toxicol. Chem.* **2006**, *25* (6), 1512−1521.
(54) Escher, B. I.; Baumer, A.; Bittermann, K.; Henneberger, L.; König, M.; Kühnert, C.; Klüver, N. General baseline toxicity QSAR for nonpolar, polar and ionisable chemicals and their mixtures in the bioluminescence inhibition assay with Aliivibrio fischeri. *Environmental Science: Processes & Impacts* **2017**, *19* (3), 414−428.
(55) Baumer, A.; Bittermann, K.; Klüver, N.; Escher, B. I. Baseline toxicity and ion-trapping models to describe the pH-dependence of bacterial toxicity of pharmaceuticals. *Environmental Science: Processes & Impacts* **2017**, *19* (7), 901−916.
(56) Alharbi, H. A.; Saunders, D. M.; Al-Mousa, A.; Alcorn, J.; Pereira, A. S.; Martin, J. W.; Giesy, J. P.; Wiseman, S. B. Inhibition of ABC transport proteins by oil sands process affected water. *Aquat. Toxicol.* **2016**, *170*, 81−88.
(57) Armitage, J. M.; Erickson, R. J.; Luckenbach, T.; Ng, C. A.; Prosser, R. S.; Arnot, J. A.; Schirmer, K.; Nichols, J. W. Assessing the bioaccumulation potential of ionizable organic compounds: Current knowledge and research priorities. *Environ. Toxicol. Chem.* **2017**, *36* (4), 882−897.
(58) Swigert, J. P.; Lee, C.; Wong, D. C.; White, R.; Scarlett, A. G.; West, C. E.; Rowland, S. J. Aquatic hazard assessment of a commercial sample of naphthenic acids. *Chemosphere* **2015**, *124*, 1−9.
(59) Marentette, J. R.; Frank, R. A.; Bartlett, A. J.; Gillis, P. L.; Hewitt, L. M.; Peru, K. M.; Headley, J. V.; Brunswick, P.; Shang, D.; Parrott, J. L. Toxicity of naphthenic acid fraction components extracted from fresh and aged oil sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (Pimephales promelas) embryos. *Aquat. Toxicol.* **2015**, *164*, 108−117.
(60) économiques, O. d. c. e. d. d. *Test No. 236: Fish Embryo Acute Toxicity (FET) Test*. OECD Publishing: 2013.
(61) Series, E. P. *Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dubia*; Environment Canada: Ottawa, Ontario, 2007.
(62) Bartlett, A. J.; Frank, R. A.; Gillis, P. L.; Parrott, J. L.; Marentette, J. R.; Brown, L. R.; Hooey, T.; Vanderveen, R.; McInnis, R.; Brunswick, P. Toxicity of naphthenic acids to invertebrates: Extracts from oil sands process-affected water versus commercial mixtures. *Environ. Pollut.* **2017**, *227*, 271−279.
(63) CCME, A., *Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life*; Canadian Council of Ministers of the Environment: Winnipeg, Manitoba, 2007.
(64) ECHA, M. Guidance on information requirements and chemical safety assessment. *Chapter R* **2008**, *8*.
(65) Stephan, C. E.; Mount, D. I.; Hansen, D. J.; Gentile, J.; Chapman, G. A.; Brungs, W. A. *Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses*; U.S. Environmental Protection Agency: Duluth, MN, 1985.
(66) Team, R. C. R. *A language and environment for statistical computing*, 2013.
(67) Bittermann, K.; Spycher, S.; Goss, K.-U. Comparison of different models predicting the phospholipid-membrane water partition coefficients of charged compounds. *Chemosphere* **2016**, *144*, 382−391.

DOI: 10.1021/acs.est.8b00614
Environ. Sci. Technol. 2018, 52, 8039−8049

(68) Redman, A. D.; Parkerton, T. F.; Leon Paumen, M.; Butler, J. D.; Letinski, D. J.; den Haan, K. A re-evaluation of PETROTOX for predicting acute and chronic toxicity of petroleum substances. *Environ. Toxicol. Chem.* **2017**, *36*, 2245.
(69) Armstrong, S. A.; Headley, J. V.; Peru, K. M.; Germida, J. J. Phytotoxicity of oil sands naphthenic acids and dissipation from systems planted with emergent aquatic macrophytes. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2007**, *43* (1), 36−42.
(70) Rendal, C.; Kusk, K. O.; Trapp, S. Optimal choice of pH for toxicity and bioaccumulation studies of ionizing organic chemicals. *Environ. Toxicol. Chem.* **2011**, *30* (11), 2395−2406.
(71) Frank, R. A.; Sanderson, H.; Kavanagh, R.; Burnison, B. K.; Headley, J. V.; Solomon, K. R. Use of a (quantitative) structure−activity relationship [(Q) Sar] model to predict the toxicity of naphthenic acids. *J. Toxicol. Environ. Health, Part A* **2009**, *73* (4), 319−329.
(72) Haftka, J. J.-H.; Hammer, J.; Hermens, J. L. Mechanisms of neutral and anionic surfactant sorption to solid-phase microextraction fibers. *Environ. Sci. Technol.* **2015**, *49* (18), 11053−11061.
(73) McGrath, J. A.; Fanelli, C. J.; Di Toro, D. M.; Parkerton, T. F.; Redman, A. D.; Paumen, M. L.; Comber, M.; Eadsforth, C. V.; den Haan, K. Re-evaluation of Target Lipid Model-Derived HC5 Predictions for Hydrocarbons. *Environ. Toxicol. Chem.* **2018**, *37*, 1579.
(74) Wezel, A. P. Chemical and biological aspects of ecotoxicological risk assessment of ionizable and neutral organic compounds in fresh and marine waters: a review. *Environ. Rev.* **1998**, *6* (2), 123−137.

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Supplemental Information

Application of the target lipid model and passive samplers to characterize the toxicity of bioavailable organics in oil sands process-affected water

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***Tables found in accompanying spreadsheet.**

Detailed methods used in toxicity testing and chemical analysis of exposure solutions

Zebrafish (D. rerio) embryo toxicity tests

An outbred strain of wild-type zebrafish obtained from Aquatic Research Organisms (Hampton, NH), was used in this study. Studies were conducted according to OECD protocol 236 [1]. Embryos were exposed in 22 mL GC vials, capped with Teflon-coated screw caps, and placed into an environmental chamber at 28 ± 1 °C. Samples of water were taken from each treatment for quantification of test substances on study day 0, representing "new" solutions. Additional water samples were taken at termination of the test on day 4, representing "old" solutions, and were composites of treatment replicates to provide sufficient volume for extraction. Water quality parameters (e.g., pH, dissolved oxygen, conductivity) were within guideline specifications.

Microscopic observations were performed at 24 ± 1 h intervals. Endpoints recorded included number of coagulated eggs, lack of somite formation, and tail-bud detachment from yolk sac. Following the first 24 h observation, the remaining endpoint observed was lack of heartbeat. Mortality was determined by coagulation or absence of heartbeat.

Ceriodaphnia dubia 3-brood reproduction assay

C. dubia were cultured in reconstituted moderately hard water and were supplied by Aquatic Research Organisms (Hampton, NH, USA). According to the ECCC test guideline [2], individual *C. dubia* were maintained in 20 mL glass scintillation vial filled entirely with test solution and sealed with a Teflon-lined screw cap. Culture chambers were maintained at 25 ± 2 °C under a 16 :8 h light:dark. Exposure waters were renewed daily. Samples of water were taken from each treatment solution for analysis of test substance concentrations on study days 0 and 5 representing "new" solutions, prior to

exposures with the test organism. Additional samples of water were taken on study days 1 and 6 representing "old" solutions following exposures of test organisms, and composites of treatment replicates were used to provide sufficient volume for extraction. Cultures and test organisms were fed a suspension of algae (*Pseudokirchnerella subcapitata*) ($2.6x10^5$cells/mL) and yeast and trout chow, added to each renewal test chamber daily prior to organism transfer. Water quality parameters were within guideline specifications.

Observations for immobilization were performed and recorded at approximately 24 h intervals following test initiation. Immobilization was defined as a lack of swimming within 15 seconds after gentle agitation of the test container. Adults were transferred daily while neonates were enumerated at each daily renewal of the exposure solution. The presence of aborted eggs and immobilized offspring were also recorded. Following test completion, the total number of living offspring produced per living adult at the end of the test was assessed.

Preparation of toxicity test solutions

A concentrated stock solution of each model compound was prepared two days before initiating tests and a dilution of each treatment level was made on the day of initiation. pH was measured prior to addition of animals during each daily renewal of exposure solutions. Where appropriate, adjustments to pH were made in the stock solution to raise the pH to 7.3 (+/- 0.2) one day before initiation. Treatments and controls were prepared and mixed at test temperature with Teflon®-coated stir bars and stirred throughout the study.

Defined mixtures of individual test chemicals were prepared to test additivity. This was done by preparing a mixture of six individual chemicals, each at 1/6 of their observed EC10 for *C. dubia* or LC50 for *D. rerio* (Table S2). Chemicals studied were cyclohexylbutyric, decahydronaphthalene-2-carboxylic, trimethylcyclohexyl acetic, trimethyladamantane carboxylic, cyclopentanoic, and undecanoic acids. Test data were not available for all of the individual compounds on *D. rerio*, so for chemicals that were tested on both species, exposure concentrations were scaled by the relative difference between toxic potencies to fish and daphnids (Table 2).

Exposure solutions for some poorly soluble chemicals, including tridecanoic and cholanic acids, were developed by use of a glass generator column. Approximately one gram of each of the three solid test substances was packed into separate glass generator columns (approximate dimensions 20 cm length x 6 mm id). Test substances were retained in columns with plugs of glass wool. A "control" column was established in parallel containing only glass wool plugs. The tops (inlets) of the inner reservoirs were connected to a pump and a flow of approximately 0.8 mL/min was established. The test water (column eluent) was moderately hard (~80 mg/L as calcium carbonate) reconstituted water. Columns were maintained at a mean temperature of 25.6 °C in an environmental chamber. Eluates from columns (test solutions) were collected in 1 L glass bottles. These compounds were tested at a single concentration at the solubility limit.

Analytical confirmation of test substance exposure concentrations

Conventional analysis

All buffers, acids, and residue-grade solvents were obtained from J.T. Baker (VWR, Center Valley, PA). Single water sample replicates from the control and each

106 exposure concentration were extracted by use of solid phase extraction (SPE) by use of 1
107 mL (100 mg) octadecyl (C_{18}) J.T. Baker Bakerbond cartridges (VWR). Cartridges were
108 conditioned with methanol and laboratory glass distilled water prior to use. Samples were
109 first diluted to a final volume of 25 mL in 0.01 M pH 2.4 phosphate buffer. Diluted,
110 acidified samples were also spiked with 50 to 150 µL of 200 to 2000 µg/mL internal
111 standard solution containing a series of the non-target carboxylic acids n-decanoic acid,
112 *trans*-4-butylcyclohexanecarboxylic acid, and 4-pentylbicyclo [2.2.2] octane-1-
113 carboxylic acid. Cartridges were air-dried under vacuum and then eluted with 2 mL of
114 1% formic acid in methanol for more water soluble constituents or methylene chloride for
115 the more hydrophobic organic acid test compounds. Extracts were then exchanged into
116 hexane and adjusted to a final volume of 3 mL.
117 Sample extracts were quantified by use of gas chromatography, with flame
118 ionization detection (GC-FID) on a Perkin Elmer Autosystem XL gas chromatograph
119 with a 15 m x 0.53 mm id, 1.0 µm DB-FFAP stationary phase (Agilent / J&W Scientific,
120 Wilmington, DE) analytical column. For the tests with sparingly soluble chemicals that
121 were conducted with one treatment level at their solubility limit, a 15 m x 0.53 mm id,
122 1.5 µm film Rtx-5 (Restek, Belefonte, PA) analytical column was used. Twenty
123 microliter injections were made in the programmable split/splitless mode (large volume
124 injection) with the injection temperature programmed from 65 ^{o}C to 250 ^{o}C. Standards
125 and sample extracts were all injected in duplicate. The FID temperature was 250 °C and
126 initial signal attenuation setting was -4, then set to -3 at 9.60 minutes. The oven
127 temperature was held at 55 ^{o}C for 2 min and then ramped to 240 ^{o}C at 20 ^{o}C/min. The
128 column flow was 15 mL/min.

Supplemental Figures

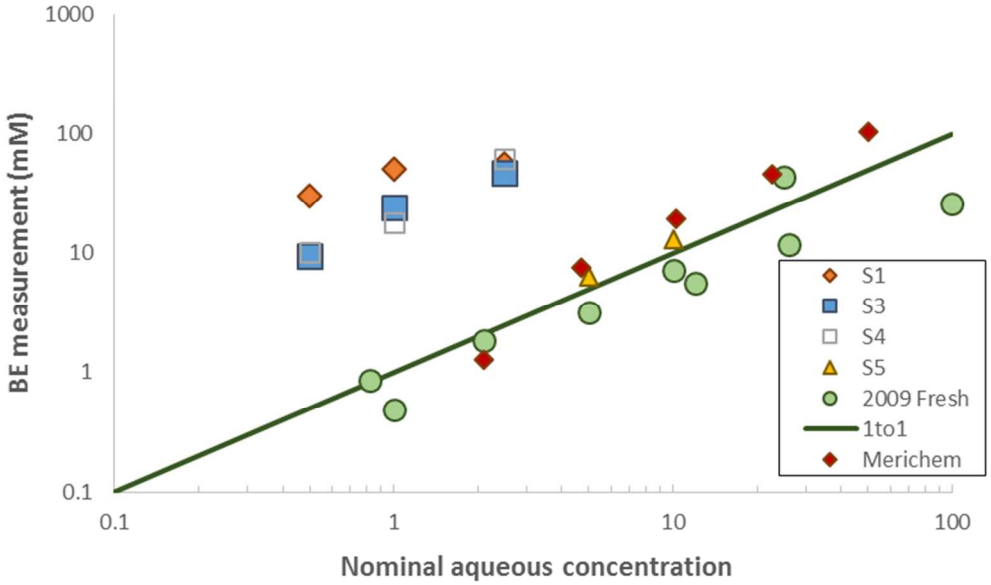

Figure S1. Comparing concentration-responses between biomimetic extraction (BE) measurements and nominal aqueous concentrations of extracts evaluated in the present study. Fraction S1, S3, S4, S5 are from Morandi et al. [3] and are reported as fold-increase over ambient concentrations. "2009 Fresh" data are from an oil sands process-affected water (OSPW) extract studied in Marentette et al. [4] and Bartlett et al [5], and the Merichem commercial naphthenic acid (NA) mixture data are from Swigert et al. [6] and are reported in "mg/L". BE measurements are generally linear with nominal aqueous concentrations, though offset depending on the substance. Therefore, the mean ratio of BE/aqueous concentration was used to extrapolate BE measurements to effects data in Tables 1 and S3 (with the exception of substance "S1", which is a residual material with limited solubility at the highest concentration and was not used in the extrapolations).

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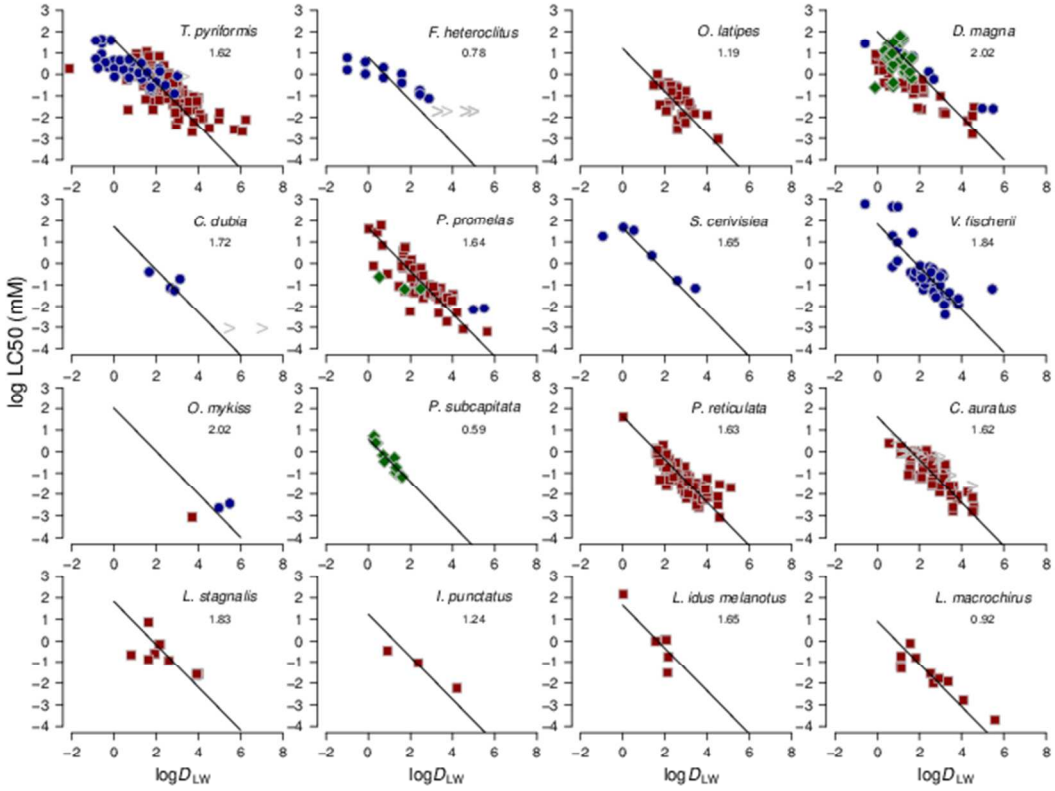


145

146 Figure S2. Comparing log LC50s reported in the literature at test pH to log D_{LW}

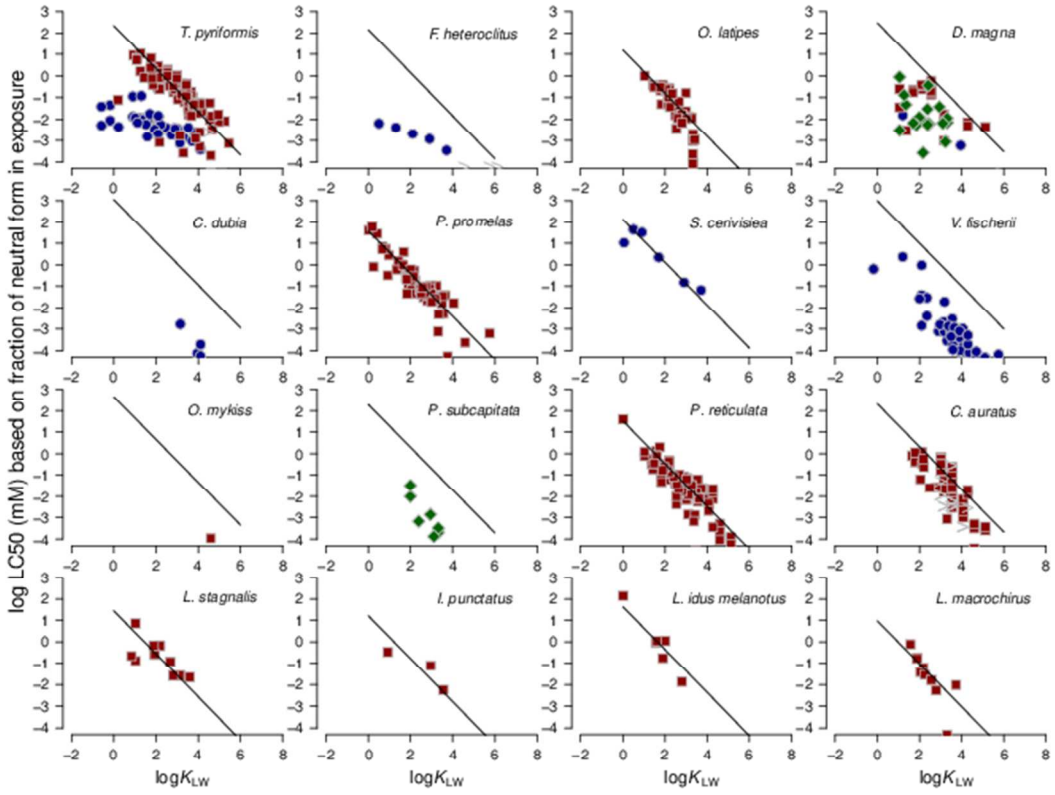
147 (Equation 2-7) . Species names and computed critical target lipid body burdens are

148 printed in each panel. Blue circles are naphthenic acid (NA) structures, red squares are

149 substituted phenols, and green diamonds are benzoic acid structures. Line is the target

150 lipid model (TLM) fit (Equation 7).

151



152

153 Figure S3. Comparing the fraction of the LC50 that is in the neutral form (based on pH in

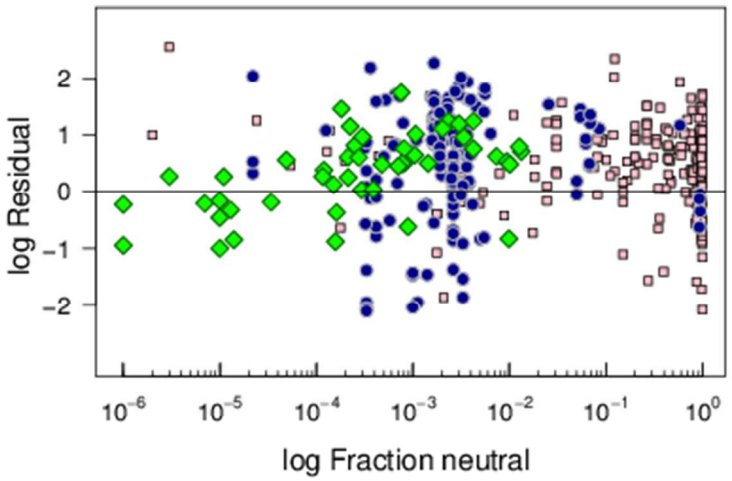
154 the test system and pKa) to log K_{LW} of the neutral form of the chemical for each species

155 in the database. Blue circles are naphthenic acid (NA) structures, red squares are

156 substituted phenols and green diamonds are benzoic acid structures. Line is the target

157 lipid model (TLM) fit in Figure S2.

158



159

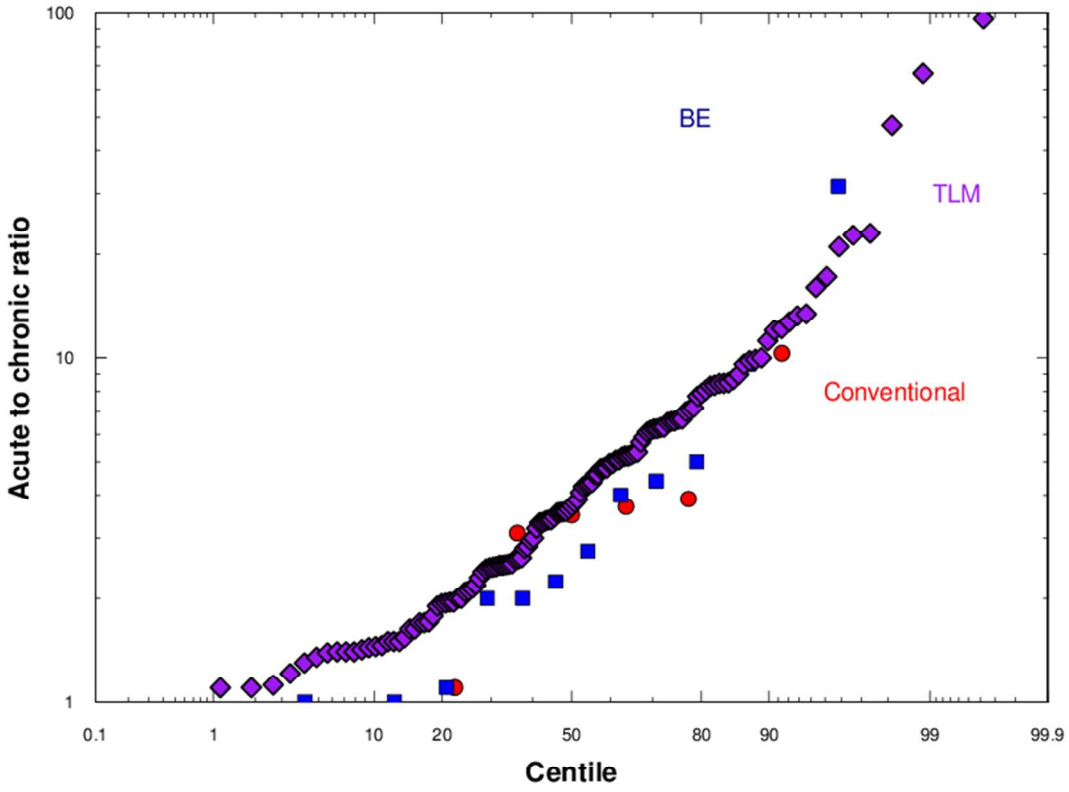
160 Figure S4. Log residuals (Predicted LC50/Observed LC50) for data in Figure S2

161 compared to the computed fraction of chemical in the neutral form based on the test pH

162 and pKa. Blue circles are naphthenic acid (NA) structures, red squares are substituted

163 phenols, and green diamonds are benzoic acid structures.

164



165

166 Figure S5. Distribution of Acute to Chronic Ratio (ACR) for nonpolar organics from the
167 target lipid model (TLM) database (purple diamonds) [7], and ACRs from the experimental
168 work in this study based on either conventional metrics (red circles, e.g., LC50/EC10 in
169 mg/L in water) or based on the BE measurements (blue squares, e.g., LC50/EC10 in
170 mmol/L of polydimethylsiloxane (PDMS) resin on the solid phase microextraction
171 (SPME) fiber).

172

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References

1. OECD, *Test No. 236: Fish Embryo Acute Toxicity (FET) Test*. OECD Publishing: 2013.
2. Series, E. P., Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dubia. In Enviro.
3. Morandi, G. D.; Wiseman, S. B.; Pereira, A.; Mankidy, R.; Gault, I. G.; Martin, J. W.; Giesy, J. P., Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. *Environmental science & technology* **2015,** *49*, (20), 12395-12404.
4. Marentette, J. R.; Frank, R. A.; Bartlett, A. J.; Gillis, P. L.; Hewitt, L. M.; Peru, K. M.; Headley, J. V.; Brunswick, P.; Shang, D.; Parrott, J. L., Toxicity of naphthenic acid fraction components extracted from fresh and aged oil sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (Pimephales promelas) embryos. *Aquatic Toxicology* **2015,** *164*, 108-117.
5. Bartlett, A. J.; Frank, R. A.; Gillis, P. L.; Parrott, J. L.; Marentette, J. R.; Brown, L. R.; Hooey, T.; Vanderveen, R.; McInnis, R.; Brunswick, P., Toxicity of naphthenic acids to invertebrates: Extracts from oil sands process-affected water versus commercial mixtures. *Environmental Pollution* **2017,** *227*, 271-279.
6. Swigert, J. P.; Lee, C.; Wong, D. C.; White, R.; Scarlett, A. G.; West, C. E.; Rowland, S. J., Aquatic hazard assessment of a commercial sample of naphthenic acids. *Chemosphere* **2015,** *124*, 1-9.
7. Redman, A. D.; Parkerton, T. F.; Paumen, M. L.; McGrath, J. A.; den Haan, K.; Di Toro, D. M., Extension and validation of the target lipid model for deriving predicted no□effect concentrations for soils and sediments. *Environmental Toxicology and Chemistry* **2014,** *33*, (12), 2679-2687.