Environmental Science & Technology

Article

pubs.acs.org/est

Effect of Lipid Partitioning on Predictions of Acute Toxicity of Oil Sands Process Affected Water to Embryos of Fathead Minnow (*Pimephales promelas*)

Garrett D. Morandi,[†] Kun Zhang,[‡] Steve B. Wiseman,[†] Alberto dos Santos Pereira,[‡] Jonathan W. Martin,[‡] and John P. Giesy*[,†,§,∥,⊥,#]

[†]Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A2, Canada

[‡]Division of Analytical and Environmental Toxicology, University of Alberta, Edmonton, Alberta Canada

[§]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A2, Canada

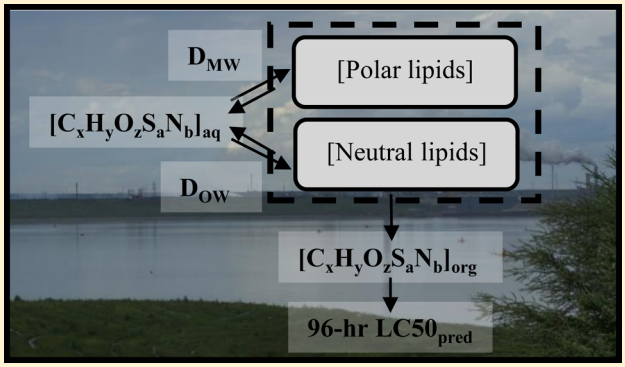
[∥]Department of Zoology, and Center for Integrative Toxicology, Michigan State University, East Lansing, Michigan 48823, United States

[⊥]School of Biological Sciences, University of Hong Kong, Hong Kong, SAR China

[#]State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, People's Republic of China

Supporting Information

ABSTRACT: Dissolved organic compounds in oil sands process affected water (OSPW) are known to be responsible for most of its toxicity to aquatic organisms, but the complexity of this mixture prevents use of traditional bottom-up approaches for predicting toxicities of mixtures. Therefore, a top-down approach to predict toxicity of the dissolved organic fraction of OSPW was developed and tested. Accurate masses (i.e., m/z) determined by ultrahigh resolution mass spectrometry in negative and positive ionization modes were used to assign empirical chemical formulas to each chemical species in the mixture. For each chemical species, a predictive measure of lipid accumulation was estimated by stir-bar sorptive extraction (SBSE) to poly-(dimethyl)siloxane, or by partitioning to solid-supported lipid membranes (SSLM). A narcosis mode of action was assumed and the target-lipid model was used to estimate potencies of mixtures by assuming strict additivity. A model developed using a combination of the SBSE and SSLM lipid partitioning estimates, whereby the accumulation of chemicals to neutral and polar lipids was explicitly considered, was best for predicting empirical values of LC50 in 96-h acute toxicity tests with embryos of fathead minnow (*Pimephales promelas*). Model predictions were within 4-fold of observed toxicity for 75% of OSPW samples, and within 8.5-fold for all samples tested, which is comparable to the range of interlaboratory variability for in vivo toxicity testing.



INTRODUCTION

The oil sands regions of northern Alberta, Canada, contain among the largest proven reserves of petroleum in the world. Oil sands process affected water (OSPW) is a byproduct of extraction of bitumen from oil sands and is a mixture of residual hydrocarbons, silts, clays, and dissolved organic and inorganic constituents.[1,2] OSPW which is acutely and chronically toxic to a range of organisms is stored in tailings ponds during the active life of oil sands surface mines.[2,3] When surface mines are closed, or when OSPW is no longer required for extraction of bitumen, OSPW must be remediated,[4] and ultimately must be hydraulically reconnected with the natural environment. To this end, the end-pit lake (EPL) strategy designates previously mined-out areas for long-term storage and remediation of process affected materials, including OSPW.[3] Over time, natural degradation within EPLs is hoped to attenuate toxicity of OSPW. Base Mine Lake (BML) was established in 2012 and is the first commercial scale test of the EPL strategy.

OSPW is a complex mixture of organic compounds[5,6] containing naphthenic acids (NAs), oxidized NAs and related organic acids containing sulfur or nitrogen, as well as nonacidic polar neutral substances.[5,7−10] Because natural in situ aging or treatment of OSPW by activated charcoal adsorption or ozonation significantly attenuates, or removes, all toxic effects of OSPW, it is accepted that the dissolved organic fraction is

Received: March 25, 2016
Revised: June 28, 2016
Accepted: July 15, 2016
Published: July 15, 2016

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

responsible for most acute toxicity to aquatic organisms.[11−16] Advances in identification of dissolved organic compounds in OSPW, by ultrahigh resolution mass spectrometry (uHRMS), has improved understanding of its composition. By measurement of accurate mass (i.e., m/z) in both positive (+) and negative (−) ionization modes, this technique facilitates identification of chemical "species" based on empirical formulas, and binning of these species into broader "heteroatomic classes" sharing the same numbers of heteroatoms (i.e., oxygen, sulfur and nitrogen).[7−9]

The specific organic compounds responsible for the acute toxicity of OSPW has been the focus of much research.[15,17−19] It has long been reported that NAs (O_2^- empirical formula class, general formula $C_nH_{2n+z}O_2$, where z represents the number of hydrogen atoms absent due to rings or double bonds[20]) are the primary agents causing acute toxicity.[17,21−24] This assumption is further supported by elevated concentrations of O_2^- containing chemical species OSPW and in fractions of OSPW with acute toxicity.[24,25] However, recently by use of a bioassay effects-directed analysis (EDA) for BML OSPW it was demonstrated that in addition to NAs, other heteroatomic classes also contribute to acute toxicity of OSPW (i.e., O^-, SO_2^-, O^+, O_2^+, SO^+, and NO^+).[25] Some of these nonacidic chemical classes also showed high predicted propensity to accumulate by use of stir-bar sorptive extraction (SBSE) to poly(dimethyl)siloxane, or by partitioning to solid-supported lipid membranes (SSLM).[26,27]

Narcosis, a reversible mode of toxic action, has been suggested as the mode of acute toxicity of OSPW, in part because organic extracts of OSPW demonstrate steep dose−response relationships similar to other narcotic chemicals.[19,24,25,28] It is accepted that the onset of toxic effects of narcotic chemicals is related to an aquatic species-specific concentration in lipid, termed the critical body burden (C_{bb}).[29] Toxic potencies of narcotic chemicals are related to their potential to accumulate in lipids, specifically, it is the volume of the molecules dissolved in lipids, especially the phospholipid bilayer of membranes. Once dissolved in the membrane narcotic molecules disrupt a range of processes including membrane fluidity, gap-junction cell−cell communication and activities of membrane-bound enzymes.[31] Traditionally, the tendency of a molecule, specifically a neutral molecule, to partition into these lipids has been described by use of the chemical independent parameter, octanol−water partition coefficient (K_{OW}).[29−32] To this end, the Critical Target Lipid Body Burden (CTLBB) model has been developed to predict toxicity of narcotic chemicals to a wide range of species by use of a linear free energy relationship (LFER) that relates toxicity to K_{OW} and a species' C_{bb} (eq 1).[33] In this equation, LC50 is the concentration required to cause 50% mortality (mmol/L), m is the universal narcosis slope developed by Van Leeuwen et al.,[34] K_{OWi} is a compound-specific bioaccumulation potential, Δc is a chemical class specific correction factor, and b is an aquatic species specific C_{bb} (eq 1).

$$\log(\mathrm{LC50}) = m\log(K_{OW}) + \Delta c + \log(b) \quad (1)$$

It is now recognized that the C_{bb} of a narcotic chemical is a function of accumulation into both neutral and polar lipids of fish.[35] Although K_{OW} accurately describes accumulation of narcotic chemicals into neutral lipids, it cannot be used to accurately assess accumulation of narcotics, particularly polar chemicals, into polar lipids, such as phospholipids, which make up cell membranes.[35,36] Improvements in predicting aquatic toxicity of polar chemicals acting by a narcosis mode of action have been made by accounting for accumulation of polar chemicals to phospholipids in addition to neutral lipids.[35,37]

Biomimetic approaches using solid sorbents facilitate prediction of potentials for compounds to be accumulated into lipids. Uptake from water by a surrogate lipid material, such as poly(dimethyl)siloxane (PDMS), measures the fraction of neutral organic compounds that is freely available for uptake into an organism. By use of previously defined relationships, measured PDMS partition coefficients (K_{PDMS}) can be used to predict K_{OW} and accumulation potential into neutral storage lipids.[38,39] In addition to neutral lipids which make up approximately 6% of tissues of fish, polar lipids account for up to 1.25% of total lipids in fish and are known to be a target for polar organic chemicals acting by a narcosis mode of action.[35−37] Extending surrogate lipid material such as PDMS to assess accumulation potential of polar organic chemicals can result in underpredictions of accumulation because it does not account for interactions of chemicals with relatively polar (and charged) phospholipids.[36,40] Solid-supported lipid membranes (SSLM), composed of a phospholipid bilayer, offer an approach which improves estimates of bioaccumulation for ionic and polar organic chemicals. Analogous to K_{OW}, the partition coefficient of a chemical between the SSLM and water, known as membrane affinity (D_{MW}), can be derived to more accurately predict potentials of such chemicals to bioaccumulate.[41,42]

Compositions of petroleum mixtures are highly variable and therefore toxicity models must be adaptable. To this end, the hydrocarbon block approach has been developed, whereby hydrocarbons in mixtures are separated by carbon number and compound class and assigned representative structures.[45] Simplification of the mixture into hydrocarbon blocks facilitates calculations and prediction of environmental fates and distributions of mixture components and by use of the CTLBB inherent toxicity of individual hydrocarbons can be estimated.[43−45] Because chemical species in OSPW might exist as a mixture of isomers,[8] measured bioaccumulation estimates can represent one or more chemical species in the mixture. Therefore, in this work the complex dissolved organic fraction of OSPW was described by use of detectable accurate masses in both positive and negative mode. Following description of the mixture, inherent toxicity of individual hydrocarbons in the aqueous phase can be estimated by use of the CTLBB assuming a narcosis mode of action.[43−45] Mixture effects can then be assessed by use of the toxic unit (TU) approach, assuming strict additivity of the hazard. By use of such approaches, relative hazards of mixtures can be assessed in a risk assessment framework.

The purpose of the present study was to develop a model to predict acute lethality of the extractable organic fraction of OSPW[25] to embryos of the model fish, fathead minnow (*Pimephales promelas*). To this end, estimates of toxic potencies of mixtures by use of chemical composition required three basic elements; identification of mixture constituents, an assessment of their concentration in the mixture, and the inherent toxic potency of each constituent for a defined end point. Therefore, in this work previously published data sets were assembled for model development and used to assess model performance. By use of existing model frameworks for predicting mixture toxicity, four models (described below in Model Parametrization) were developed to evaluate if existing model frameworks for mixture toxicity prediction can be applied to OSPW, the effect of accumulation estimates (i.e., measured pH dependent

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

octanol−water distribution ratio (D_{OW}) and D_{MW}) on toxicity predictions and to identify chemical classes contributing to the toxicity of dissolved organic chemicals in OSPW (Figure S1 of the Supporting Information, SI).

MATERIALS AND METHODS

Data Compilation. For model development, empirical acute toxicity, estimated potential to accumulate in lipids, and chemical characterization data of samples were compiled from an EDA of dissolved organic chemicals in OSPW by Morandi et al.,[25] and two studies by Zhang et al.[26,27] Because the primary goal of the EDA approach is to isolate active compounds or chemical classes, it inherently produces samples (fractions) which can be used to test the accuracy and specificity of the model. Therefore, the model presented here is complementary to the EDA approach and can be used to further understand contributions of certain chemical classes to the acute toxicity of OSPW, to assess potential critical mechanisms of toxic action of OSPW, and make predictions of acute lethality to aquatic organisms. The EDA fractions used for model development were produced previously[25] and correspond to samples generated following three rounds of sequential fractionation and toxicity testing. Therefore, samples from each round of fractionation can be identified by their first two letters (Ex: samples preceded by F1- correspond to samples generated during round 1 fractionation). Estimates of D_{OW} and D_{MW} for each species in OSPW were taken from Zhang et al.,[26,27] where a D_{OW} or D_{MW} was not reported for a certain species detected in OSPW, estimates were made by use of chemical class-specific regressions for groups of chemical species, relating log D_{OW} or log D_{MW} to molecular mass. Information on number of measured and predicted values for each sample are presented in the Tables S1 and S2.

Sample Characterization. The profile of dissolved organic chemicals in samples was determined by use of high pressure liquid chromatography with Orbitrap uHRMS detection (Orbitrap Elite, Thermo Fisher Scientific, San Jose, CA, U.S.A.) as described by Pereira et al.[8] Details of the analytical method are provided in the SI. In this study, individual species identified by use of Orbitrap uHRMS were tracked and referred to as individual chemical species by use of their accurate mass and molecular formula in each ionization mode as described by Pereira et al.[8] for the O_2^- and O_2^+ formula classes (i.e., a distinct empirical formula detected in negative mode was named separately from the same empirical formula detected in positive mode). Detected species were assigned to bins based on heteroatomic empirical formula class in negative (−) or positive (+) ionization modes: O_x (where x = 1−6), NO_x (where x = 1−4), SO_x (where x = 1−4), or NO_xS (where x = 1−2).

Constituent Concentrations. Models of toxicity for exposure of aquatic organisms to petroleum hydrocarbons employ a multicompartment fate model to predict environmental distributions of constituents of mixtures, followed by an assessment of potential effects of the aqueous phase.[45,46] However, because the mixture of interest was the extractable dissolved organic phase of OSPW filtrate (1.2 μm), the distribution of chemical species that were detected was assumed to be 100% in the aqueous phase, thus simplifying model assumptions. Therefore, concentration of an individual species (i) of a particular sample (j) was calculated by use of eqs 2 and 3. Where $RI_{i,j}$ is the relative intensity of species i of sample j calculated as the intensity of species i,j ($I_{i,j}$) over the sum of the responses of all species detected in sample j (eq 2). Concentrations of species i,j ($Cw_{i,j}$, in mmol/L) were calculated as the $RI_{i,j}$ multiplied by the gravimetric mass of organics in the sample (Mo_j) over the molecular mass of the species ($MM_{i,j}$) (eq 3). It was assumed that the total response of a sample was accounted for in the measured gravimetric mass[25] of the dried organic fraction and that individual chemical species had a response factor of 1 (1.0) in the mass spectrometer. Thus, the molar concentration of each species was defined only by its relative intensity, its molecular mass, and the total gravimetric mass of dissolved organics.

$$RI_{i,j} = \frac{I_{i,j}}{\sum I_j} \tag{2}$$

$$C\omega_{i,j} = \frac{RI_{i,j} \times Mo_j}{MW_i} \tag{3}$$

Predicted Toxic Potencies. Acute toxic potency of each chemical species was predicted assuming a narcosis mode of action by use of the CTLBB (eq 1).[33] The C_{bb} for fathead minnow, 105 μmol/g octanol, has been derived from a data set of 182 data points, consisting of a variety of chemical classes including halogenated and nonhalogenated aliphatic and aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), alcohols, ethers, furans, and ketones.[33] The LC50 of a species i ($LC50_i$) was estimated using eq 4, where the bioaccumulation potential of chemical species i (BP_i) is the chemical species specific bioaccumulation potential (D_{OW} and/or D_{MW}) and C_{bb} is the fathead minnow specific critical body burden (eq 4). Chemical class corrections (Δc) were not applied because when only the molecular formula is known it is not feasible to assign the individual species to functional group chemical classes (e.g., alcohol, ether, furan, ketone, etc.). The CTLBB has been developed by use of K_{OW} to describe the accumulation of narcotic chemicals. However, because there are recognized deficiencies in using K_{OW} to predict the accumulation and thus toxicity of polar organic chemicals,[41,42] it was instructive to investigate the use of D_{MW} in predicting the toxicity of chemical species in OSPW, many of which are known to be acidic.

$$\log(LC50)_i = mBP_i + \log(C_{bb}) \tag{4}$$

Prediction of Toxic Potencies in Mixtures. Contribution of chemical species to mixture toxicity was assessed by use of toxic units (TU) with lethality as the end point predicted. This approach normalizes the aqueous concentration of a chemical by its end point specific toxicity, the LC50 in this work (eq 5). Therefore, the relative hazard ($TU_{i,j}$) of species i,j was calculated by use of eq 5, as $Cw_{i,j}$ over $LC50_i$ (eq 5). Toxicity of the sample (TUm_j) was calculated as the sum of $TU_{i\cdots n,}$ of sample j (eq 6). When TUm_j was equal to or greater than 1, samples were expected to elicit 50% mortality or greater.

$$TU_{i,j} = \frac{C\omega_{i,j}}{LC50_i} \tag{5}$$

$$TUm_j = \sum TU_j \tag{6}$$

Model Parametrization. Because the choice of partition coefficient can affect interpretation of toxicity,[36] it was of interest to investigate the effect of BP_i on estimates of toxic potency. Therefore, four models were developed, each of which

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

Table 1. Comparison of Model Predicted LC50, and Empirical LC50 Values for Embryos of Fathead Minnow Exposed to Samples of the Extractable Dissolved Organic Fraction of OSPW[a]

sample	gravimetric mass, 100% effluent equivalent (mg/L)	predicted LC50 model I (mg/L)	predicted LC50 model II (mg/L)	predicted LC50 model III (mg/L)	predicted LC50 model IV (mg/L)	observed LC50 (mg/L)
F1-NE	50.0	32.0	76.0	76.0	23.2	36.0
F1-AE	103	1.64×10^4	1.05×10^3	1.05×10^3	990	857
F1-BE	14.0	4.14×10^3	990	1.08×10^3	280	>140
F1-Pool	167	737	242	239	181	1.33×10^3
F2-NE1	15.7	17.7	64.2	65.5	16.2	>157
F2-NE2	34.3	38.4	97.1	98.1	29.7	66.2
F2-Pool	50.0	32.7	32.0	78.0	22.6	89.5
F3-NE2a	20.0	852	104	104	92.8	14.6
F3-NE2b	14.0	31.0	110	108	28.3	30.5
F3-Pool	34.0	50.2	10.5	110	32.3	23.1
maximum		1.64×10^4	1.05×10^3	1.08×10^3	990	1.33×10^3
minimum		17.7	10.5	65.5	16.2	14.6

[a]Measured gravimetric mass of samples corresponding to its equivalent in 100% OSPW are presented as well.

used different estimates of distribution between water and organisms. The first model (Model I) estimated toxicity of mixtures by use of measured D_{OW} for each chemical species. The second model (Model II) estimated toxicity by use of D_{MW} only, and any chemical species which showed no significant partitioning ($D_{MW} < 1$) in membrane partitioning experiments were ignored by the model. The third model (Model III) was developed by use of both Model I and Model II, whereby estimates of toxicity were made by use of D_{MW} when measured data was available, in preference of D_{OW}. A fourth model (Model IV) was developed by use of all available data, assuming that chemical species with both a measured D_{OW} and D_{MW} partition into neutral and polar lipids and contribute to toxicity (Table S3).

Statistical Analysis. Statistical analyses were performed by use of SPSS software (IBM SPSS Statistics, Amtrak, NY, U.S.A.). LC50 values from Morandi et al.,[25] were normalized to the gravimetric mass (Table 1) of the respective samples. Spreadsheet models were developed by use of Excel (2013) (Microsoft Excel, Microsoft, Redmond, WAS, U.S.A.). Predictions of the four models were compared to empirical data for lethality assembled from Morandi et al.[25] Goodness-of-fit statistics, mean and median residuals were calculated as the mean or median difference between observed and predicted LC50 values, the mean absolute deviation (MAD) was calculated as the mean of the absolute value of the residuals, and the root-mean square deviation (RMSD) was calculated as the square root of the mean of the residuals squared.

RESULTS AND DISCUSSION

Model Verification. The distribution of heteroatom classes in samples are presented (Figure S2). Values of LC50 predicted by the various models are compiled (Table 1) and compared to observed LC50 values, with a line showing one-to-one correspondence (Figure 1). Observed toxicity spanned 2 orders of magnitude (14.6−1.33×10^3 mg/L), which was similar to ranges of predictions made by use of Models II, III, and IV. Predictions made by use of Model I were more variable, spanning 3 orders of magnitude (Table 1). The mean, median, log residual error, MAD, and RMSD were also compiled (Table S4). The log residual plot of Models I, II, III, and IV demonstrated no obvious deviations from the mean observed LC50 (Figure S3) and were log normally distributed (Figure S4). Because no significant lethality was observed for F1-BE and F1-NE1 samples, they were not included in the residual analysis. In general, predictions of acute lethality from each model compared well with observed toxicity and the goodness-of-fit statistics of Models II, III, and IV were similar, and were better relative to Model I.

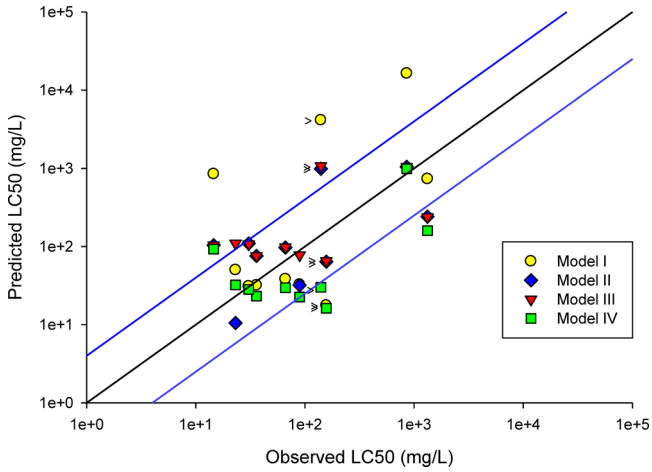


Figure 1. Comparison of model predicted LC50 to observed LC50 for embryos of fathead minnow exposed to samples of OSPW for 96 h. Yellow squares represent predictions made by use of Model I, blue diamonds represent predictions made by use of Model II, red triangles represent predictions made by use of Model III, and green squares represent predictions made by use of Model IV. The black solid line represents the line of perfect agreement, and blue solid lines represent a 2-fold error. Greater than signs identify samples which did not have observed LC50 values.

Analysis of toxicity databases has demonstrated significant interlaboratory variations among acute aquatic toxicity tests for the same chemical, experimental design, and species.[47,48] Deviations from the geometric mean for a given chemical and end point, of a factor-of 2, 5, and 10 were found to encompass 57, 86, and 94% of acute lethality results, respectively.[47] Furthermore, work by Baas et al.[48] demonstrated interlaboratory deviations for acute lethality of narcotic chemicals of 2- to 8-fold. A 2-fold difference from empirical data encompassed 50, 37.5, 25.0, and 50% of predictions by use of Models I, II, III, and IV, respectively. These results compared well with the performance of the PETROTOX model which was 42.9% for petroleum products, and is similar to the 2-fold

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

range associated with the CTLBB.[44,45] In addition, 75.0, 62.5, 75.0, and 75.0% of predictions were within a 4-fold difference of observed toxicity for Models I, II, III, and IV, respectively, and results compared well with the 69.4% value observed by Redman et al.[45] The F1-BE sample was predicted to be nontoxic within the range of tested concentrations, agreeing with the observed lack of lethality in the assay. By use of a 5-fold difference from observed LC50, the F2-NE1 sample was predicted to cause acute lethality within the range of tested concentrations but none was observed, resulting in a false positive rate for all models of 1 in 9. Using the highest tested concentration for comparison, Models I, II, III, and IV were greater than 8-, 2-, 2-, and 9-fold different, respectively (Table 1). Similarly, the false negative rate for Models I, II, III, and IV were 2, 1, 1, and 1 of 9 predictions, respectively. Toxic potency of sample F3-NE2a was under-predicted by use of all four models, but the difference from the empirical data set was less than 8.5-fold for Models II, III, and IV while Model I was different by greater than a factor-of 58. Furthermore, the toxicity of sample F1-AE was under-predicted by a factor of 19 by Model I but was within a factor of 2 for Models II, III, and IV.

A plot of predicted TU*m* and observed mortality (Figure 2) can be used to identify model inadequacies. In general, predictions of Models II, III, and IV were accurate to within a factor-of 5 compared to the empirical data set. Significant acute lethality occurred at 0.19 TU (F3-NE2a), and the 50% effect level spanned 0.140−5.54 TU (Figure 2). By use of Models II, III, and IV toxicity of the F1-Pool sample was overpredicted by a factor of 5.56, 5.48, and 8.32 respectively, while toxicity of F3-NE2a was under-predicted by a factor of 7.14 for Models II and III and 6.36 by use of Model IV. When a factor of 8.5-fold from observed LC50 was applied, all predictions of toxicity were protective, as well as being within interlaboratory variation, or accuracy of empirical tests[47,48] Predictions made with Model I were less accurate, since two samples (F1-AE and F3-NE2a) exceeded a 10-fold difference from observed effects. Significant acute lethality occurred at 0.023 TU (F3-NE2a), and the 50% effect level spanned 0.017-2.963 TU.

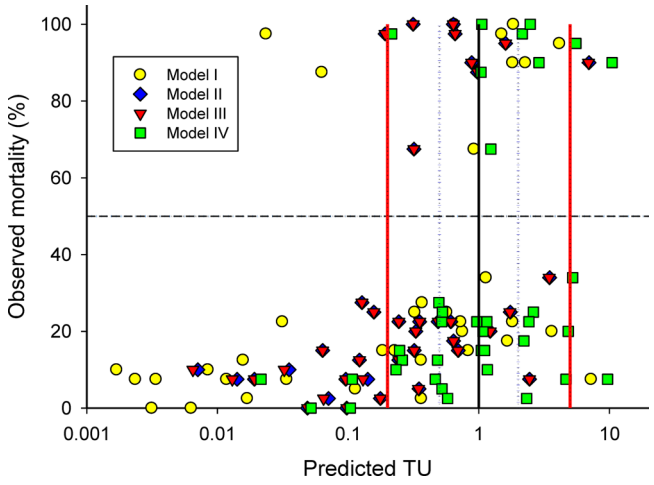


Figure 2. Comparison of model predicted toxic units (TU) to observed mortality for embryos of fathead minnow exposed to samples of OSPW. Yellow squares represent predictions made by use of Model I, blue diamonds represent predictions made by use of Model II, red triangles represent predictions made by use of Model III, and green squares represent predictions made by use of Model IV. The black solid line corresponds to 1 toxic unit, the dotted black line represents 50% mortality, blue dotted lines area 2-fold error, and red solid lines are 5-fold error.

Model Selection. As demonstrated above, differences in predicted toxicity were observed among models, and some discrepancies were found when comparing each to empirical data. For chemicals causing lethality via a narcotic mode of action, toxic potency is directly proportional to the fraction that is accumulated into the body of a particular species.[49] Therefore, the C_{bb} is aquatic species-specific and chemical independent, and the toxic potency of a compound acting through this mechanism of action is dependent on its distribution from water to the body.[30,50] To exert a toxic effect, chemicals need to be accumulated into the body, or at least interact with membranes of the gill. Accumulation of polar organic chemicals in lipid is not well predicted by use of D_{OW}, and D_{MW} is known to better describe the behavior of these chemicals.[40] Because OSPW is composed of both polar and neutral polar organic chemicals, it was instructive to investigate the effect that potential to accumulate in phospholipids had on prediction of toxicity and how explicit consideration of chemical distribution among lipid types affected accuracy of predictions of toxicity. Models II, III, and IV incorporated D_{MW} and had better goodness-of-fit statistics, accuracy, specificity, and robustness when compared to Model I (Table S4, Figures S3 and S4), which was based solely on D_{OW}. In addition, Model IV was developed assuming narcotic chemicals distribute among polar and neutral lipids, and the improved performance of this model demonstrated the utility of explicitly considering the differential accumulation among lipid types in predicting the acute toxicity of the dissolved organic fraction of OSPW.

OSPW is a mixture and its composition, to some extent, is known to be variable spatially and temporally.[51,53] Therefore, a model to predict toxicity in a given sample must be sufficiently robust to describe toxicity of varying mixtures. Although goodness-of-fit statistics for Model II and IV were similar, inclusion of all available D_{OW} and D_{MW} data into Model IV resulted in the model having greater robustness. Due to this, there is greater confidence in predictions of toxic potencies made by use of Model IV because of its increased domain of applicability and explicit assessment of chemical accumulation in polar and neutral lipids.

Contribution of Chemical Classes to Acute Lethality of BML-OSPW. Incorporation of hazard assessment frameworks into the EDA approach has previously been used to assess relative contributions of chemical classes to the toxic potency of mixtures.[44,52,55] The F1-Pool sample contains all dissolved organic compounds in BML-OSPW.[25] In the work of Morandi et al.,[25] NAs (i.e., the O_2^- class) were highlighted for their contribution to acute toxic potency of OSPW due to their large relative abundance in the most potent sample, F3-NE2a. In addition, abundant chemical classes in the sample F3-NE2b, $O^{\pm}$, O_2^+, SO^+, NO^+, and SO_2^{-+}, were cited for their contributions to acute toxic potency of BML-OSPW.[25] Therefore, it was of interest to investigate the predicted contribution of these previously identified chemical classes to the acute lethality of the F1-Pool sample by use of Model IV, which was selected as the preferred model in this work. Toxic units of the chemical classes: $O^{\pm}$, $O_2^{\pm}$, SO^+, NO^+, and SO_2^-, were summed and accounted for 97.3% of total calculated TUs in the F1-Pool sample (i.e., 97.3% of predicted toxicity for all chemicals detected in the sample), while representing less than

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

Table 2. Relative Potencies of the Chemical Classes Identified as Acutely Toxic by Morandi et al.[25a]

		percent TU (%) of F1-pool sample			
chemical class	potency (TU per percent relative intensity)	C5−15	C16−20	C21−25	C26−30
SO^+	8.13	5.79	20.3	2.59	3.25
SO_2^-	3.28	0.85	7.75	0.15	<0.01
NO^+	2.43	8.33	7.40	1.24	<0.01
$O2^-$	1.68	4.42	11.9	0.91	<0.01
$O2^+$	1.44	7.05	7.93	6.12	0.03
O^+	0.99	2.41	1.43	0.20	<0.01
O^-	4.60×10^{-4}	$<4.60 \times 10^{-4}$	$<4.60 \times 10^{-4}$	$<4.60 \times 10^{-4}$	$<4.60 \times 10^{-4}$

[a]Values were calculated as the total TU of a chemical class over its percent relative response by use of Model IV. Percent contribution of carbon number ranges to the total TU calculated for the F1-Pool sample are also presented for each chemical class.

43.4% of total mass spectral intensity. The chemical classes $O^{\pm}$, $O_2^{\pm}$, SO^+, NO^+ and SO_2^- are predicted to account for a disproportionate amount of toxicity, thereby demonstrating their combined potency relative to other chemical classes, which accounted for less than 3% of total calculated TUs and greater than 56% of total mass spectral intensity. Contributions of specific chemical classes (i.e., O_2^-, O_2^+, NO^+, SO^+, and SO_2^-) to total TU_m are displayed in Figure S5. Normalization of total calculated TU of each chemical class by its percent relative mass spectral response separates chemical classes based on their relative toxic potencies (Table 2). By use of this approach, although $O_2^{\pm}$, NO^+ and SO^+ chemical classes contributed the majority of total TU of the mixture, based on their relative intensities, the SO^+ and SO_2^- chemical classes are suggested by this result to be among the most potent toxic chemical classes in OSPW. Interestingly, the SO^+ chemical class was among the most hydrophobic chemical classes in OSPW, based on its partitioning to PDMS and SSLM, thus this class may be relatively bioaccumulative if not metablized.[26,27] In addition, analysis of TU contribution by carbon number ranges highlights contributions of chemical species with a carbon number between 16 and 20 (Table 2). Evidence presented here agrees with previous results that the $O^{\pm}$, $O_2^{\pm}$, SO^+, NO^+, and SO_2^- classes are together responsible for the majority of acute toxicty of OSPW,[25−27] and provides further evidence of the contributions from SO^+ and SO_2^-. It is also important to note the utility of the proposed model in improving our understanding of contributions of chemical classes to toxicity of a mixture. Previous work has identified NAs as the most toxic chemical species in OSPW,[25] whereas evidence here suggests that $SO^{\pm}$ and NO^+ compounds might be more potent and in some cases, contribute more to toxicity.

Limitations of Model. Deviations of predicted toxicity from observed toxicity occurred and might be related to assumptions made in development of the model. In the current study, concentrations of individual chemical species were calculated assuming a mass spectral response factor of 1 (eq 3). It is known that the response of chemicals in the OSPW matrix differ from their responses in a more simple solution,[53] and it is an oversimplification to assume that each species has the same mass spectral response per unit mass injected. Nevertheless, this approach was the only reasonable assumption that could be made with available data. Due to the complexity of OSPW (e.g., hundreds of thousands to millions of individual isomers),[53,54] identities of the majority of compounds were not known and authentic standards cannot be synthesized or purchased for the chemicals in the dissolved organic fraction.

In addition, a narcosis mode of action was assumed for predictions of toxicity. This assumption might not accurately represent the potencies of all components of the mixture, since both neutral and polar organic compounds are known to act via a number of different modes of action.[55] Previously, comparisons of observed and model predicted toxicity have been used to classify chemicals by their mode of action.[55] However, because identities and specific chemical characteristics of the majority of chemicals in OSPW are unknown, this approach could not have been taken, and comparisons can solely be drawn between whole mixture toxicity predictions and observed toxicity. Furthermore, deviations observed might be related to chemical class specific inadequacies of the CTLBB in describing toxicity and as demonstrated in previous works[31,33,56−58] the application of correction factors might improve model predictions. Due to limitations from a lack of knowledge of identities of individual chemicals in OSPW, corrections of LC50 values, such as those suggested by McCarthy et al.[50] cannot be applied. Despite assumptions made in development of the presented predictive aquatic toxicity model, predicted LC50 values did not differ by greater than 8.5-fold from empirically derived toxicity data including the most complete mixture (F1-Pool), representing the dissolved organic fraction in BML.

MODEL APPLICATION AND RELEVANCE

Currently, it is not practical to chromatographically separate and identify the structure of each organic chemical compound in OSPW. For example, recent applications of supercritical fluid chromatography demonstrate the utter complexity of isomers that can be present for various chemical species.[53] Nevertheless, models for assessment of hazards of petroleum mixtures by use of its chemical composition require identification, categorization, and representative structural assignment for all components of the mixture, followed by prediction of their environmental fate and subsequent effects. Because structures or representative structures are not known for the vast majority of chemical species in OSPW, a novel approach was developed whereby mixture components are not identified explicitly, but rather, characterized and labeled by use of their accurate masses under both negative and positive ionization. In this way, the chemicals can be binned into classes based on empirical formulas derived from the identified accurate mass. Therefore, following a simple extraction method and characterization by use of Orbitrap uHRMS, identified chemical species can be matched to bioaccumulation estimates from previously published data sets to predict the 96 h LC50 of the dissolved organic fraction of OSPW to embryos of fathead minnow (Figure S1). This approach allowed prediction of the toxicity of

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

complex mixtures with accuracies well within the range of empirical measures. In addition, the developed model was used to assess contributions of previously defined chemical classes to the toxicity of OSPW,[25] complementary to the EDA approach, and highlighted the potential contribution of SO^+ and SO_2^+ chemical classes, and chemical species with a carbon number of 16−20, to toxicity. We propose that the model developed during this study is sufficiently accurate and robust to make predictions of potential acute lethality. Furthermore, if the primary mode of toxic action of dissolved organic compounds in OSPW is narcosis, then future work should focus on the use of acute to chronic ratios to assess if the presented model can be expanded to make predictions of potential chronic toxicity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01481.

Chemicals and materials; membrane-water partition coefficient derivation; characterization of fractions by Orbitrap MS and sample profiles; conceptual flowchart, distribution of log residuals, log probability plot of residuals and relative potency of chemical classes versus relative intensity; number of available versus predicted bioaccumulation estimates, model parametrization data and error analysis (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +1-306-966-2096; fax: +1-306-966-4796; e-mail: jgiesy@aol.com (J.P.G.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The presented research was supported by a Collaborative Research and Development Grant from the Natural Science and Engineering Research Council of Canada and Syncrude Canada Ltd. to J.P.G. and J.W.M. (Project # C4288). Additional support was obtained from a Discovery Grant from the Natural Science and Engineering Research Council of Canada (Project # 326415−07) and grants from Western Economic Diversification Canada (Project # 6578 and 6807) to J.P.G. The authors acknowledge the support of an instrumentation grant from the Canada Foundation for Innovation. J.P.G. was supported by the Canada Research Chair program, and at large Chair Professorship at the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, and the Einstein Professor Program of the Chinese Academy of Sciences. The authors acknowledge the support of the Aquatic Toxicology Research Facility (ATRF) at the Toxicology Centre, University of Saskatchewan, in providing space and equipment for the culturing and breeding of fathead minnows. J.W.M. acknowledges financial support from Alberta Health for support of daily laboratory activity. The authors wish to thank Warren Zubot of Syncrude Canada Ltd. for supplying the OSPW.

■ REFERENCES

(1) MacKinnon, M. Development of the tailings pond at Syncrude's oil sands plant: 1978−1987. *AOSTRA J.Res.* **1989**, *5* (2), 109−133.

(2) Mikula, R. J.; Kasperski, K. L.; Burns, R. D.; MacKinnon, M. D. Nature and fate of oil sands fine tailings. *Adv. Chem. Ser.* **1996**, *251*, 67710.1021/ba-1996-0251.ch014

(3) Gosselin, P.; Hrudey, S. E.; Naeth, M. A.; Plourde, A.; Therrien, R.; Van Der Kraak, G.; Xu, Z. Environmental and health impacts of Canada's oil sands industry. *Royal Society of Canda.* **2010**.

(4) 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.

(5) 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.

(6) Lengger, S. K.; Scarlett, A. G.; West, C. E.; Rowland, S. J. Diamondoid diacids ('O4'species) in oil sands process-affected water. *Rapid Commun. Mass Spectrom.* **2013**, *27* (23), 2648−2654.

(7) 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.

(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.; Peru, K.; Fahlman, B.; Colodey, A.; McMartin, D. Selective solvent extraction and characterization of the acid extractable fraction of Athabasca oils sands process waters by Orbitrap mass spectrometry. *Int. J. Mass Spectrom.* **2013**, *345*, 104−108.

(10) Jones, D.; Scarlett, A.; West, C.; Frank, R.; Gieleciak, R.; Hager, D.; Pureveen, J.; Tegelaar, E.; Rowland, S. Elemental and spectroscopic characterization of fractions of an acidic extract of oil sands process water. *Chemosphere* **2013**, *93* (9), 1655−1664.

(11) He, Y.; Wiseman, S. B.; Zhang, X.; Hecker, M.; Jones, P. D.; El-Din, M. G.; Martin, J. W.; Giesy, J. P. Ozonation attenuates the steroidogenic disruptive effects of sediment free oil sands process water in the H295R cell line. *Chemosphere* **2010**, *80* (5), 578−584.

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

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

(14) Anderson, J.; Wiseman, S.; Wang, N.; Moustafa, A.; Perez-Estrada, L.; Gamal El-Din, M.; Martin, J.; Liber, K.; Giesy, J. P. Effectiveness of ozonation treatment in eliminating toxicity of oil sands process-affected water to Chironomus dilutus. *Environ. Sci. Technol.* **2012**, *46* (1), 486−493.

(15) Klamerth, N.; Moreira, J.; Li, C.; Singh, A.; McPhedran, K. N.; Chelme-Ayala, P.; Belosevic, M.; El-Din, M. G. Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. *Sci. Total Environ.* **2015**, *506*, 66−75.

(16) Scott, A. C.; Zubot, W.; MacKinnon, M. D.; Smith, D. W.; Fedorak, P. M. Ozonation of oil sands process water removes naphthenic acids and toxicity. *Chemosphere* **2008**, *71* (1), 156−160.

(17) Verbeek, A.; Mackay, W.; MacKinnon, M. Isolation and characterization of the acutely toxic compounds in oil sands process water from Syncrude and Suncor. *Oil sands: Our petroleum future conference*. 1993, *25*(04).

(18) Lo, C. C.; Brownlee, B. G.; Bunce, N. J. Mass spectrometric and toxicological assays of Athabasca oil sands naphthenic acids. *Water Res.* **2006**, *40* (4), 655−664.

(19) 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.

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

(20) 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.
(21) MacKinnon, M. D.; Boerger, H. Description of two treatment methods for detoxifying oil sands tailings pond water. *Water Qual. Res. J. Can.* **1986**, *21* (4), 496−512.
(22) Verbeek, A.; Mackay, W.; MacKinnon, M. Acute toxicity of oil sands wastewater: A toxic balance. *Proceedings of the twentieth annual aquatic toxicity workshop*. 1994, *26*(13).
(23) Nero, V.; Farwell, A.; Lister, A.; Van Der Kraak, G.; Lee, L.; Van Meer, T.; MacKinnon, M.; Dixon, D. Gill and liver histopathological changes in yellow perch (Perca flavescens) and goldfish (Carassius auratus) exposed to oil sands process-affected water. *Ecotoxicol. Environ. Saf.* **2006**, *63* (3), 365−377.
(24) Scarlett, A.; Reinardy, H.; Henry, T.; West, C.; Frank, R.; Hewitt, L.; Rowland, S. Acute toxicity of aromatic and non-aromatic fractions of naphthenic acids extracted from oil sands process-affected water to larval zebrafish. *Chemosphere* **2013**, *93* (2), 415−420.
(25) Morandi, G. D.; Wiseman, S.; Pereira, A. D. S.; Mankidy, R.; Gault, I. G.; Martin, J. W.; Giesy, J. P. Effects-Directed Analysis of Dissolved Organic Compounds in Oil Sands Process Affected Water (OSPW). *Environ. Sci. Technol.* **2015**, *49* (20), 12395−404.
(26) 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.
(27) Zhang, K.; Wiseman, S. B.; 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.
(28) Nobels, I.; Spanoghe, P.; Haesaert, G.; Robbens, J.; Blust, R. Toxicity ranking and toxic mode of action evaluation of commonly used agricultural adjuvants on the basis of bacterial gene expression profiles. *PLoS One* **2011**, *6* (11), e2413910.1371/journal.-pone.0024139
(29) McCarty, L.; Mackay, D.; Smith, A.; Ozburn, G.; Dixon, D. Interpreting aquatic toxicity QSARs: The significance of toxicant body residues at the pharmacologic endpoint. *Sci. Total Environ.* **1991**, *109*, 515−525.
(30) Abernethyand, S.; Mackay, D.; McCarty, L. Volume fraction" correlation for narcosis in aquatic organisms: The key role of partitioning. *Environ. Toxicol. Chem.* **1988**, *7* (6), 469−481.
(31) McCarty, L. S.; Mackay, D. Enhancing ecotoxicological modeling and assessment. Body residues and modes of toxic action. *Environ. Sci. Technol.* **1993**, *27* (9), 1718−1728.
(32) McCarty, L.; Dixon, D.; MacKay, D.; Smith, A.; Ozburn, G. Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: Neutral narcotic organics. *Environ. Toxicol. Chem.* **1992**, *11* (7), 917−930.
(33) McCarty, L.; Dixon, D.; Ozburn, G.; Smith, A. Toxicokinetic modeling of mixtures of organic chemicals. *Environ. Toxicol. Chem.* **1992**, *11* (7), 1037−1047.
(34) 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.
(35) Van Leeuwen, C. J.; Van Der Zandt; Peter, T. J.; Aldenberg, T.; Verhaar, H. J.; Hermens, J. L. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. I. Narcotic industrial pollutants. *Environ. Toxicol. Chem.* **1992**, *11* (2), 267−282.
(36) van Wezel, A. P.; Punte, S. S.; Opperhuizen, A. Lethal body burdens of polar narcotics: Chlorophenols. *Environ. Toxicol. Chem.* **1995**, *14* (9), 1579−1585.
(37) Vaes, W. H.; Ramos, E. U.; Verhaar, H. J.; Hermens, J. L. Acute toxicity of nonpolar versus polar narcosis: Is there a difference? *Environ. Toxicol. Chem.* **1998**, *17* (7), 1380−1384.
(38) Verbruggen, E. M.; Vaes, W. H.; Parkerton, T. F.; Hermens, J. L. Polyacrylate-coated SPME fibers as a tool to simulate body residues and target concentrations of complex organic mixtures for estimation of baseline toxicity. *Environ. Sci. Technol.* **2000**, *34* (2), 324−331.
(39) Leslie, H. A.; ter Laak, T. L.; Busser, F. J.; Kraak, M. H.; Hermens, J. L. Bioconcentration of organic chemicals: Is a solid-phase microextraction fiber a good surrogate for biota? *Environ. Sci. Technol.* **2002**, *36* (24), 5399−5404.
(40) Mayer, P.; Vaes, W. H.; Wijnker, F.; Legierse, K. C.; Kraaij, R.; Tolls, J.; Hermens, J. L. Sensing dissolved sediment porewater concentrations of persistent and bioaccumulative pollutants using disposable solid-phase microextraction fibers. *Environ. Sci. Technol.* **2000**, *34* (24), 5177−5183.
(41) Vaes, W. H.; Urrestarazu Ramos, E.; Hamwijk, C.; van Holsteijn, I.; Blaauboer, B. J.; Seinen, W.; Verhaar, H. J.; Hermens, J. L. Solid phase microextraction as a tool to determine membrane/water partition coefficients and bioavailable concentrations in in vitro systems. *Chem. Res. Toxicol.* **1997**, *10* (10), 1067−1072.
(42) Loidl-Stahlhofen, A.; Hartmann, T.; Schöttner, M.; Röhring, C.; Brodowsky, H.; Schmitt, J.; Keldenich, J. Multilamellar liposomes and solid-supported lipid membranes (TRANSIL): screening of lipid-water partitioning toward a high-throughput scale. *Pharm. Res.* **2001**, *18* (12), 1782−1788.
(43) Loidl-Stahlhofen, A.; Schmitt, J.; Nöller, J.; Hartmann, T.; Brodowsky, H.; Schmitt, W.; Keldenich, J. Solid-Supported Biomolecules on Modified Silica Surfaces—A Tool for Fast Physicochemical Characterization and High-Throughput Screening. *Adv. Mater.* **2001**, *13* (23), 1829−1834.
(44) 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.
(45) McGrath, J. A.; Parkerton, T. F.; Hellweger, F. L.; Di Toro, D. M. Validation of the narcosis target lipid model for petroleum products: Gasoline as a case study. *Environ. Toxicol. Chem.* **2005**, *24* (9), 2382−2394.
(46) Di Toro, D. M.; McGrath, J. A.; Stubblefield, W. A. Predicting the toxicity of neat and weathered crude oil: Toxic potential and the toxicity of saturated mixtures. *Environ. Toxicol. Chem.* **2007**, *26* (1), 24−36.
(47) Pereira, A.; Martin, J. Exploring the complexity of oil sands process-affected water by high efficiency supercritical fluid chromatography/orbitrap mass spectrometry. *Rapid Commun. Mass Spectrom.* **2015**, *29* (8), 735−744.
(48) McGrath, J. A.; Parkerton, T. F.; Hellweger, F. L.; Di Toro, D. M. Validation of the narcosis target lipid model for petroleum products: Gasoline as a case study. *Environ. Toxicol. Chem.* **2005**, *24* (9), 2382−2394.
(49) Raimondo, S.; Jackson, C. R.; Barron, M. G. Influence of taxonomic relatedness and chemical mode of action in acute interspecies estimation models for aquatic species. *Environ. Sci. Technol.* **2010**, *44* (19), 7711−7716.
(50) Baas, J.; Spurgeon, D.; Broerse, M. A simple mechanistic model to interpret the effects of narcotics. *SAR QSAR Environ. Res.* **2015**, *26* (3), 165−180.
(51) Baer, K. N. Fundamentals of aquatic toxicology: effects, environmental fate, and risk assessment. *Int. J. Toxicol.* **1996**, *15* (5), 453−454.
(52) McCarty, L.; Mackay, D.; Smith, A.; Ozburn, G.; Dixon, D. Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: polar narcotic organics. *Ecotoxicol. Environ. Saf.* **1993**, *25* (3), 253−270.
(53) Lengger, S. K.; Scarlett, A. G.; West, C. E.; Frank, R. A.; Hewitt, L. M.; Milestone, C. B.; Rowland, S. J. Use of the distributions of adamantane acids to profile short-term temporal and pond-scale spatial variations in the composition of oil sands process-affected waters. *Environmental Science: Processes & Impacts* **2015**, *17* (8), 1415−1423.
(54) Rowland, S. J.; West, C. E.; Scarlett, A. G.; Ho, C.; Jones, D. Differentiation of two industrial oil sands process-affected waters by two-dimensional gas chromatography/mass spectrometry of diamondoid acid profiles. *Rapid Commun. Mass Spectrom.* **2012**, *26* (5), 572−576.

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

(55) McGrath, J. A.; Di Toro, D. M. 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.
(56) 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.
(57) 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.
(58) Russom, C. L.; Bradbury, S. P.; Broderius, S. J.; Hammermeister, D. E.; Drummond, R. A. Predicting modes of toxic action from chemical structure: acute toxicity in the fathead minnow (Pimephales promelas). *Environ. Toxicol. Chem.* **1997**, *16* (5), 948−967.

DOI: 10.1021/acs.est.6b01481
Environ. Sci. Technol. 2016, 50, 8858−8866

Supporting information

Effect of lipid partitioning on predictions of acute toxicity of oil sands process affected water to embryos of fathead minnow (*Pimephales promelas*).

Garrett D. Morandi[1], Kun Zhang[2], Steve B. Wiseman[1], Alberto dos Santos Pereira[2], Jonathan Martin[2], John P. Giesy[1,3,4,5,6,]*

[1] Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada
[2] Division of Analytical and Environmental Toxicology, University of Alberta, Edmonton, AB, Canada
[3] Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada
[4] Department of Zoology, and Center for Integrative Toxicology, Michigan State University, East Lansing, MI, USA
[5] School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China
[6] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China

Enclosed materials

Number of pages: 10

Number of figures: 5

Number of tables: 4

* Corresponding author:

Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada

Tel.: +1-306-966-2096

Fax: +1-306-966-4796

Email: jgiesy@aol.com

Materials and Methods:

Chemicals and materials. Acetic acid, dichloromethane (DCM), methanol (HPLC grade) and water (Optima grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The sample of OSPW was collected on the site of Syncrude Canada, Ltd. (Fort McMurray, Alberta, Canada) from the West-in-Pit active settling basin, now known as Base Mine Lake (BML), in March 2011 and was stored at 4 °C until use.

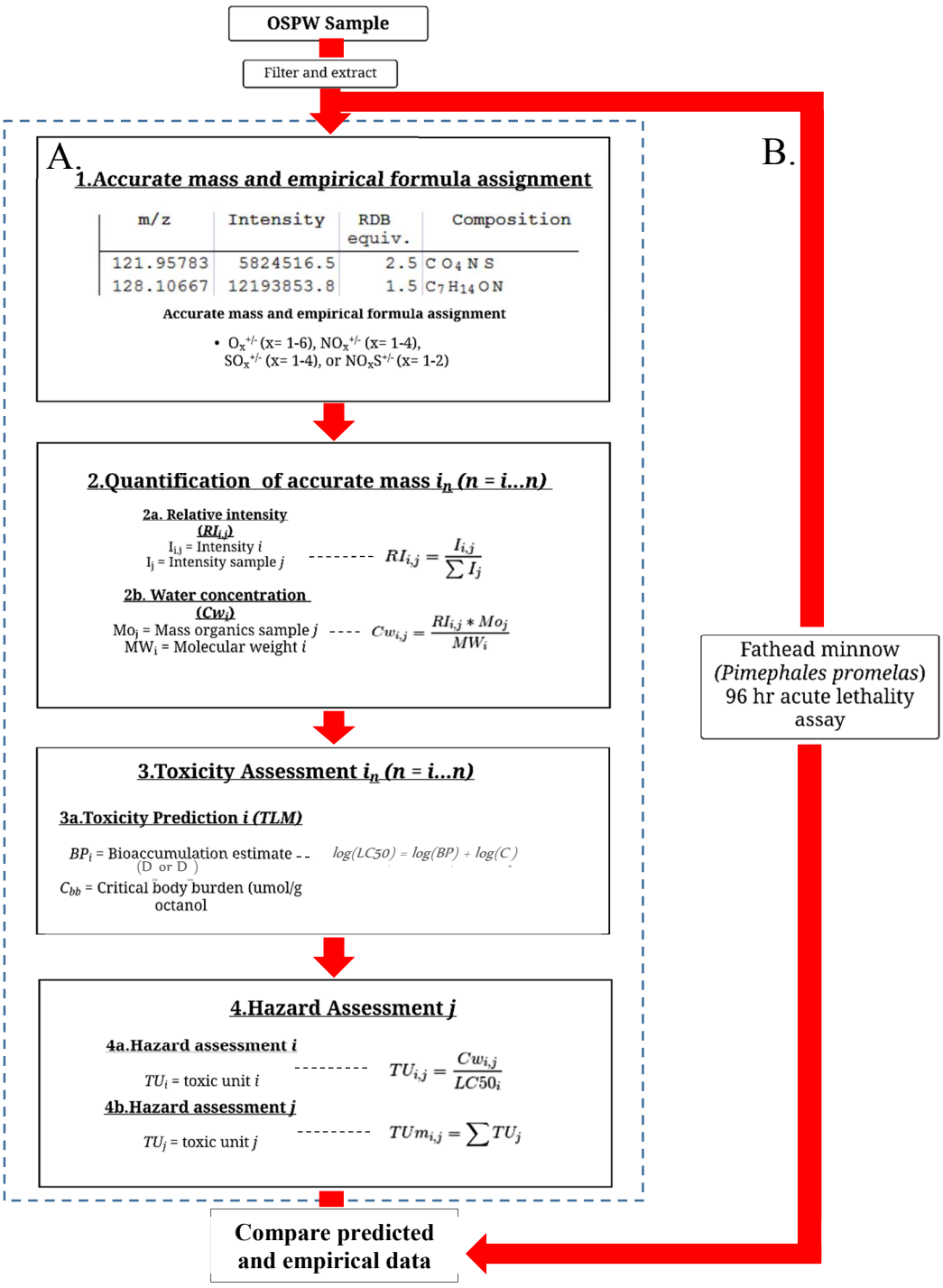
Stock preparation for derivation of membrane- water partition coefficients.

A total of 1 L of OSPW was filtered through a 0.45 μm filter (Millipore, Billerica, MA) to remove suspended solids and then extracted with 2×200 mL of DCM. Next, the extract was evaporated to near dryness with a rotary evaporator (model R- 210, Buchi, Toronto, Ontario, Canada). The remaining volume was transferred to a 20 mL glass vial and taken to full dryness under a gentle stream of nitrogen at room temperature (Turbovap LV, Biotage, Charlotte, NC) and dissolved in 1 mL of dimethyl sulfoxide (DMSO) to make stock solutions that were 1000-fold more concentrated than the original sample of OSPW. Then, 16 μL of the 1000-fold stock solutions were diluted by use of the aqueous buffer included in the membrane affinity kit to prepare stock solutions that were 50-fold more concentrated than the original sample of OSPW.

Characterization of Fractions by HPLC-Orbitrap-uHRMS.

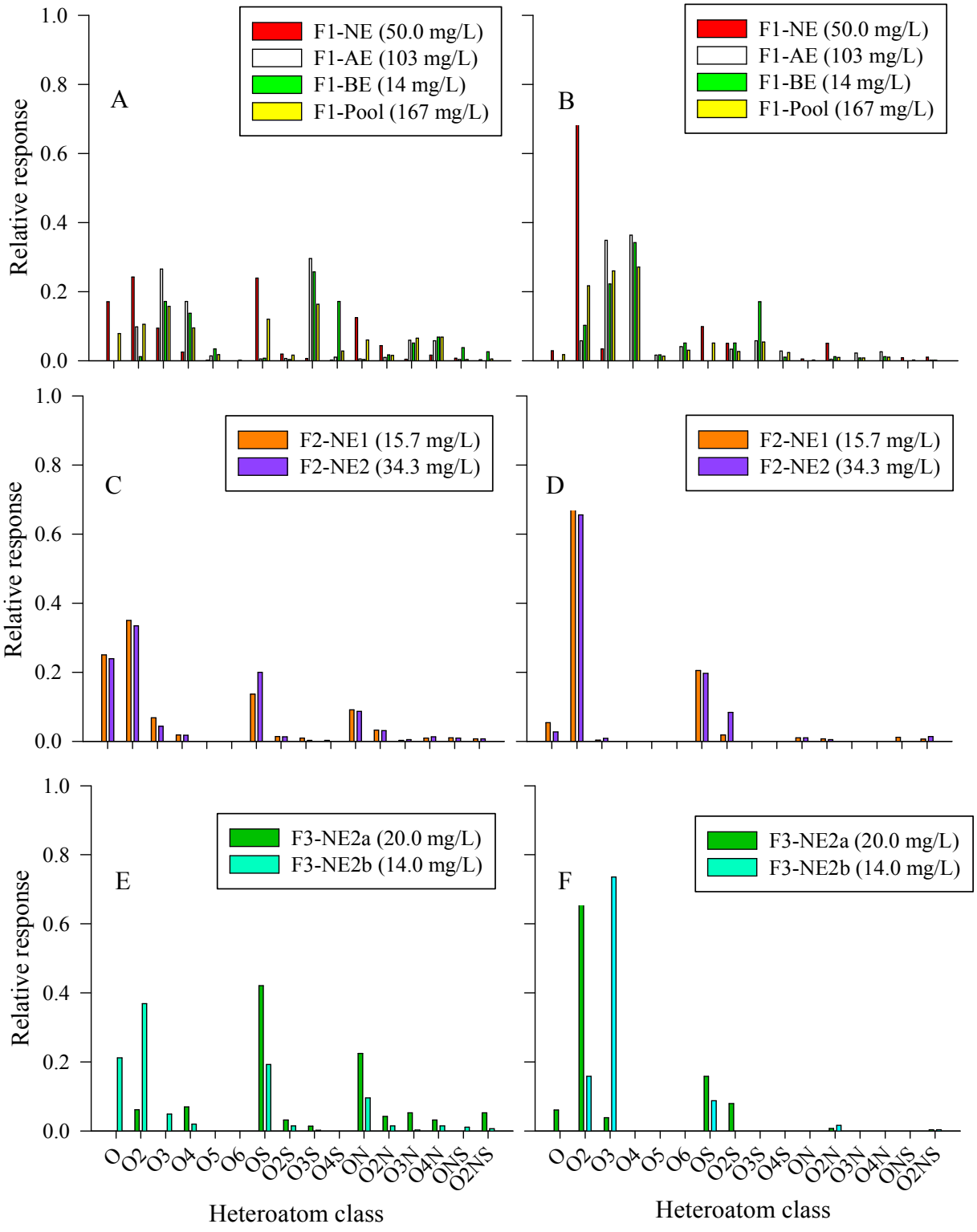
Profiles of relative proportions of organic compounds in fractions were determined by use of LC-UHRMSaccording to the method described by Pereira et al (2013). Chromatographic separation was performed by use of an HPLC Transcend system (Thermo Fisher Scientific), consisting of a degasser, a 1250 bar quaternary pump, an auto-sampler, and a column oven. Separation was performed on a Cosmosil C18 MS-II column (100 x 3.0 mm, 2.5 μm particle size) (Nacalai

54 USA, San Diego, CA, USA) at 40 °C. A flow rate of 0.5 mL/min and an injection volume of 3
55 µL were used in all analyses. Mobile phases consisted of (A) 0.1% acetic acid in water, and (B)
56 100% methanol. The mobile phase composition was 5% B for 1 min, followed by a linear
57 gradient ramp to 90% B at 9 min, to 99% B over 5 min, and returning to 5% B in 1 min followed
58 by a 4 min hold prior to the next injection.

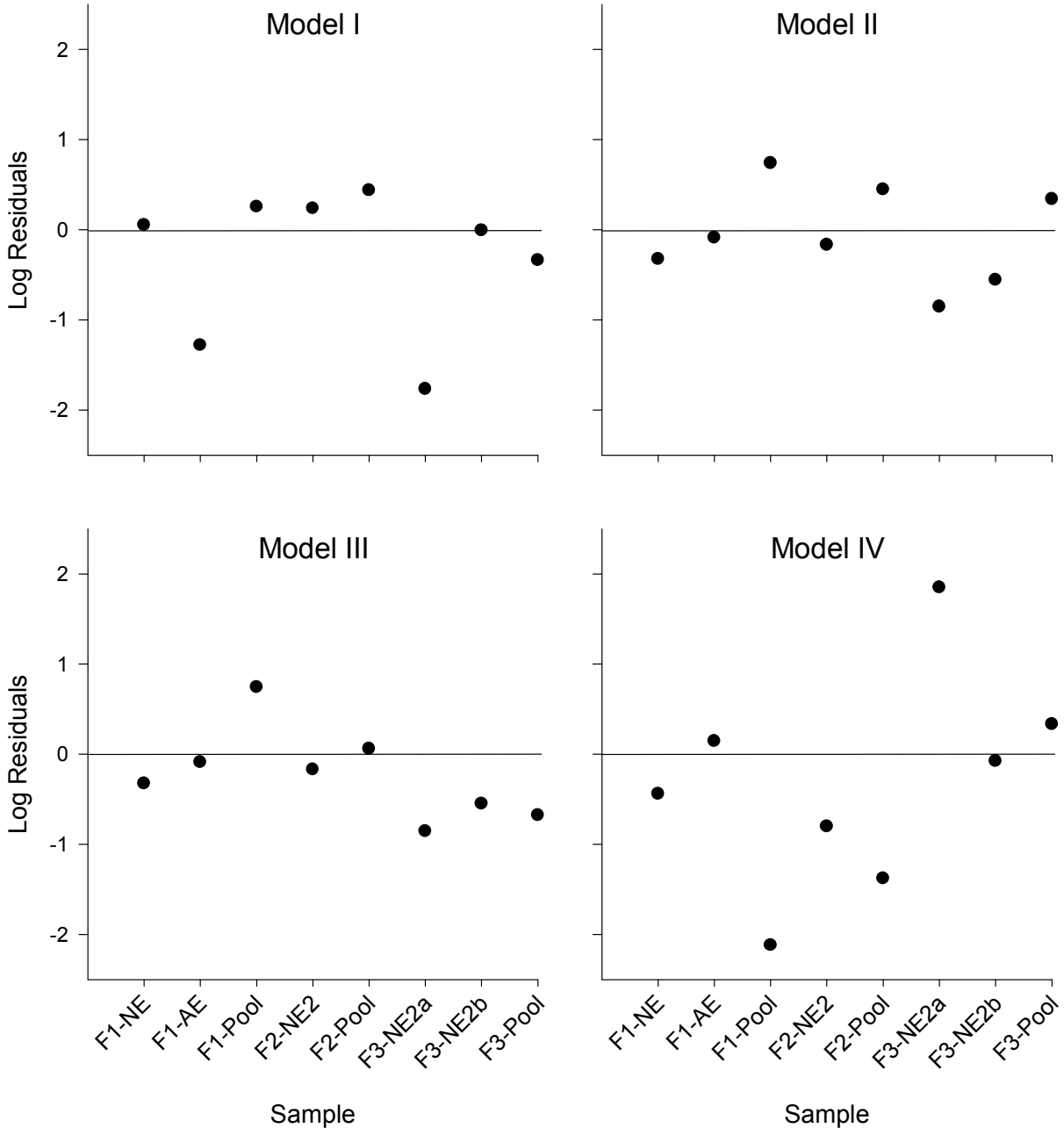


SI Figure 1. Flow chart outlining A) steps for predicting the 96 hr lethal concentration to elicit a 50% response (LC50); 1) Accurate mass detection and empirical formula assignment by use of ultrahigh resolution orbitrap mass spectrometry, 2) Calculation of water concentration of detected accurate masses as a function of relative response normalized to sample organic mass, 3) Assignment of measured or predicted bioaccumulation estimates to accurate masses assembled from Zhang et al.,[26,27] and prediction of inherent potency by use of CTLBB, 4) Hazard assessment of sample by use of the TU approach assuming strict additivity of the hazard. B)

Verification of the aquatic toxicity model, for embryos of fathead minnow exposed to extractable organics from OSPW.

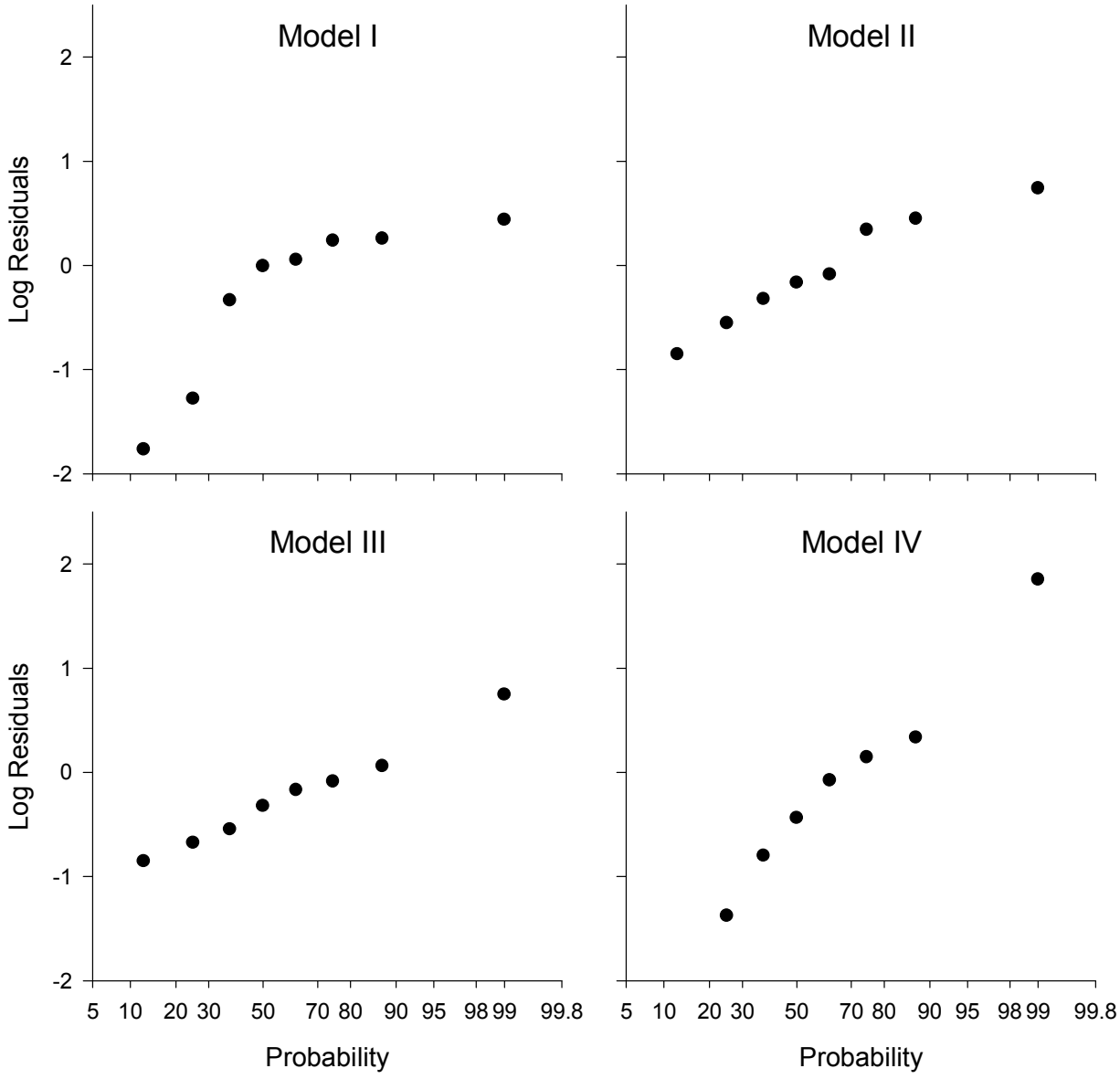


SI Figure 2. Total abundances of species by class of heteroatoms, based on sum of peak areas in chromatograms of fractions of BML-OSPW: A) Primary fractions in ESI+, B) Primary fractions in ESI-, C) Secondary fractions in ESI+, D) Secondary fractions in ESI-, E) Tertiary fractions in ESI+, F) Tertiary fractions in ESI-. Abundances were normalized to F1-Pool.



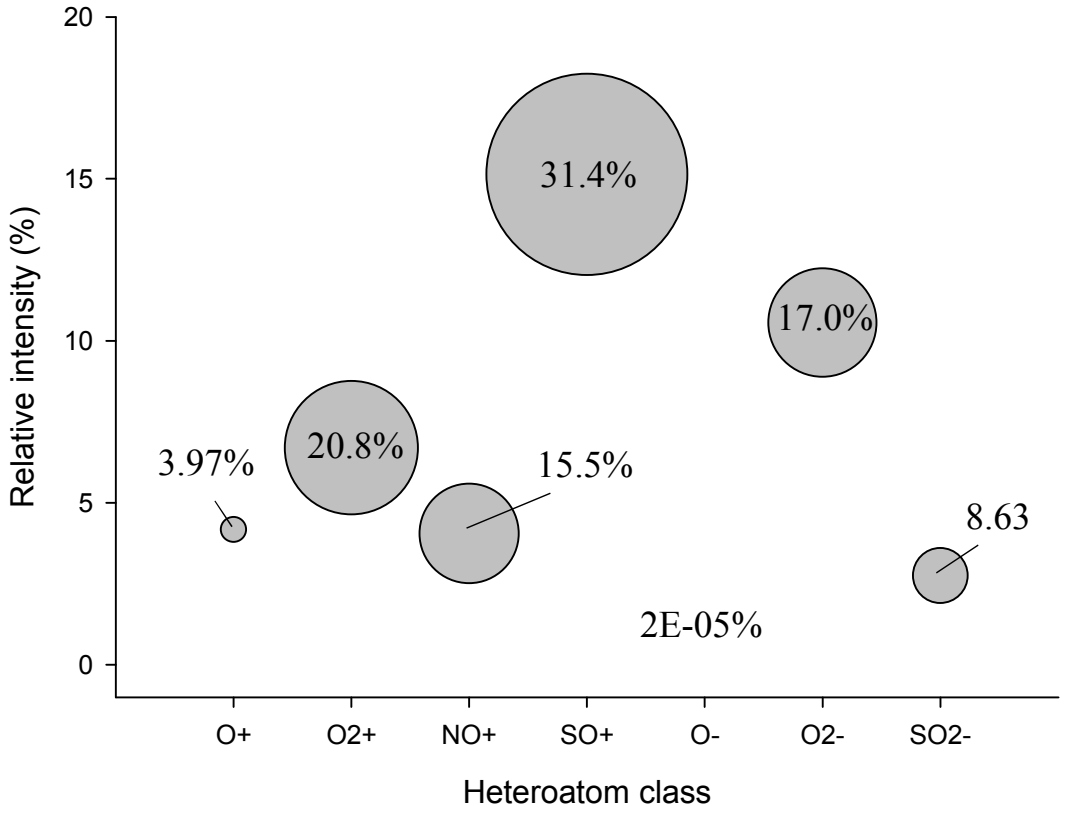
SI Figure 3. Distribution of log of residuals between predicted and observed LC50 by use of Model I, II, III and IV.

79



80

81 **SI Figure 4.** Log residuals as a function of Log probability of occurring for Model I, II, III and
82 IV.



SI Figure 5. Percent contribution of total toxic units (TU) of the chemical classes in the F1-Pool sample identified by Morandi et al. (2015)[25] plotted as a function of relative intensity. Relative intensity was calculated as the total mass spectral intensity of a chemical class over the sum of the responses of all chemical species detected in sample. Size of a circle corresponds to the percent contribution to total TU of a chemical class, O^- class did not contribute a high percentage to be visible.

SI Table 1. Number of chemical species detected in each sample, the number of measured D_{OW} values available from Zhang et al.,[26] matched to chemical species detected in samples of OSPW and number of predicted D_{OW} values for each sample of OSPW.

Sample	Number chemical species detected in sample (ESI+/-)	Number chemical species with measured Dow	Number chemical species with predicted Dow
F1-Pool	2051	1580	471
F1-NE	1208	971	237
F1-AE	1171	1041	130
F1-BE	508	420	88
F2-NE1	785	535	250
F2-NE2	824	598	226
F2-Pool	1609	1133	476
F3-NE2a	498	320	178
F3-NE2b	660	470	190
F3-Pool	1158	790	368

SI Table 2. Number of chemical species detected in each sample, the number of measured D_{MW} values available from Zhang et al.,[27] matched to chemical species detected in samples of OSPW and number of predicted D_{MW} values for each sample of OSPW.

Sample	Number chemical species detected in sample (ESI+/-)*	Number chemical species with measured D_Mw	Number chemical species with predicted D_Mw
F1-Pool	1035	269	766
F1-NE	727	175	552
F1-AE	172	59	113
F1-BE	136	35	101
F2-NE1	485	124	361
F2-NE2	505	153	352
F2-Pool	990	277	713
F3-NE2a	290	108	182
F3-NE2b	389	98	291
F3-Pool	679	206	473

SI Table 3. Bioaccumulation estimates used for toxicity predictions in the development of Model I, II, III and IV.

Model	Octanol-water distribution ratio (D_{OW})	Phospholipid membrane-water distribution ratio (D_{MW})
I	X	-
II	-	X
III*	X	X
IV**	X	X

*Toxicity estimates were made by use of D_{MW} when measured data was available, in preference of D_{OW}. **Toxicity estimates were made by use of both D_{OW} and D_{MW} when measured data was available.

SI Table 4. Calculated mean residual, median residual, mean absolute deviation (MAD) and root mean square deviation (RMSD) between predicted and observed LC50 for Model I, II, III and IV.

	Mean residual	Median residual	MAD	RMSD
Model I	0.69	-0.05	3.74	3.44
Model II	0.13	0.29	4.30	1.35
Model III	0.53	0.57	3.90	1.45
Model IV	-0.31	-0.26	4.74	1.35