Environmental Toxicology and Chemistry—Volume 40, Number 11—pp. 3000–3009, 2021
Received: 8 February 2021 | Revised: 9 March 2021 | Accepted: 15 August 2021

Environmental Chemistry

Application of the Target Lipid Model to Assess Toxicity of Heterocyclic Aromatic Compounds to Aquatic Organisms

Joy McGrath,[a] Gordon Getzinger,[b] Aaron D. Redman,[c,d] Melanie Edwards,[e] Alberto Martin Aparicio,[d] and Eleni Vaiopoulou[d,*]

[a]ERM, New York, New York, USA
[b]Exponent, Maynard, Massachusetts, USA
[c]ExxonMobil Petroleum and Chemical, Machelen, Belgium
[d]Concawe, Brussels, Belgium
[e]Integral Consulting, Seattle, Washington, USA

Abstract: Heterocyclic aromatic compounds can be found in crude oil and coal and often co-exist in environmental samples with their homocyclic aromatic counterparts. The target lipid model (TLM) is a modeling framework that relates aquatic toxicity to the octanol–water partition coefficient (K_{OW}) that has been calibrated and validated for hydrocarbons. A systematic analysis of the applicability of the TLM to heterocyclic aromatic compounds has not been performed. The objective of the present study was to compile reliable toxicity data for heterocycles and determine whether observed toxicity could be successfully described by the TLM. Results indicated that the TLM could be applied to this compound class by adopting an empirically derived coefficient that accounts for partitioning between water and lipid. This coefficient was larger than previously reported for aromatic hydrocarbons, indicating that these heterocyclic compounds exhibit higher affinity to target lipid and toxicity. A mechanistic evaluation confirmed that the hydrogen bonding accepting moieties of the heteroatoms helped explain differences in partitioning behavior. Given the TLM chemical class coefficient reported in the present study, heterocyclic aromatics can now be explicitly incorporated in TLM-based risk assessments of petroleum substances, other products, or environmental media containing these compounds. *Environ Toxicol Chem* 2021;40:3000–3009. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Heterocyclic; Polycyclic aromatic compounds; Toxicity; Target lipid model; Oil; Aromatic

INTRODUCTION

Petroleum is a complex mixture of chemicals whose toxicity is dependent on composition. PetroTox and PetroRisk are models that respectively predict hazard and risk associated with petroleum substances based on composition using the hydrocarbon block method (HBM; King et al., 1996). PetroTox is a three-phase (i.e., air, water, oil) distribution model that computes the concentration of oil components in each phase in a controlled laboratory system (Redman et al., 2012; Serrano et al., 2020) in which the predicted aqueous concentration is used to assess toxicity to aquatic organisms. PetroRisk is a multimedia exposure model used to perform risk assessments of complex petroleum substances that accounts for emissions to air, water, and soil during various stages of the material's life cycle that was developed to help stakeholders respond to requirements under the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation EC 1907/2006 (European Commission, 2006) for performing environmental risk assessments of petroleum substances (Redman et al., 2014). Both PetroTox and PetroRisk use the target lipid model (TLM) to estimate the toxicity and risk of the oil-related hydrocarbons and are publicly available on the Concawe website (n.d.).

The TLM is a quantitative structure–activity model that describes the relationship between a chemical's acute toxicity and octanol–water partition coefficient (K_{OW}; Di Toro et al., 2000) that has been validated for predicting the toxicity of petroleum hydrocarbons (McGrath et al., 2005; McGrath & Di Toro, 2009). Aromatic hydrocarbons found in petroleum may contain one aromatic ring (i.e., monocyclic aromatic hydrocarbons [MAHs]) or two or more fused rings (i.e., polycyclic aromatic hydrocarbons

This article includes online-only Supporting Information.

* Address correspondence to eleni.vaiopoulou@concawe.eu
Published online 18 August 2021 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5194

[PAHs]). The MAHs and PAHs that have the same atom in the aromatic ring, namely, carbon (e.g., benzene, pyrene) are homocyclic compounds. If a nitrogen (N), sulfur (S), or oxygen (O) is substituted for a carbon atom in the aromatic ring, these compounds are referred to as heterocyclic aromatic compounds (HACs). Heterocyclic aromatic compounds can be found in crude oil and coal and often co-exist in environmental samples with PAHs (Schwarz et al., 2014). Furans, benzothiophenes, quinolines, benzofurans, and azaarenes are examples of HACs associated with petroleum (Quann, 1998). Benzothiophenes are often measured during oil spill monitoring and for toxicity assessment (Anderson et al., 2014; Harman et al., 2009; O'Shaughnessy et al., 2018). Whereas the TLM has been widely applied to homocyclic aromatics, its applicability to heterocyclic aromatics has been limited (Redman & Parkerton, 2015). In the present study, HACs are assumed to act via the same mechanism of action as their homocyclic counterparts, namely, narcosis, and the applicability of the TLM framework for these compounds is evaluated.

The HACs are more polar and subsequently more soluble than their homocyclic counterparts and can be bioavailable in environmental media. These compounds have been measured in a variety of environmental matrices, and studies have reported effects on aquatic organisms (Bleeker et al., 1998; Brendel et al., 2018; Dijkman et al., 1997; Peddinghaus et al., 2012). Presumably, due to their lower concentrations in the environmental in comparison with their homocyclic counterparts, these heterocyclic polycyclic aromatic compounds (PACs) are not considered to be risk drivers. However, recent literature on risk assessments of PACs indicates there is a need to include the heterocyclic compounds in a risk assessment framework (Brendel et al., 2018; Hodson et al., 2020; Schwarz et al., 2014). The present study provides the hazard framework to evaluate the validity of this assumption and the need to develop a mechanistic based approach to characterize the toxicity of the heterocyclic PACs.

THE TARGET LIPID MODEL

Many nonionic organic chemicals (NIOCs) exhibit a narcotic mode of toxic action (i.e., narcosis; van Wezel & Opperhuizen, 1995). Although the exact mechanism of toxicity by narcosis is not well known, the target lipid (e.g., sensitive lipid membranes) of an organism is assumed to be the site of action. This assumption is based on the well-known correlation between the aqueous exposure concentration of a chemical needed to induce an effect (e.g., the concentration causing mortality to 50% of the test organisms [LC50]) and the chemical's K_{OW}, a metric of the degree of a chemical's hydrophobicity (McCarty et al., 1991; van Wezel & Opperhuizen, 1995; Veith et al., 1983). The TLM describes this relationship and presents a method for deriving water quality criteria for NIOCs that exert acute toxicity via narcosis (Di Toro et al., 2000). To extrapolate the acute effect concentration to a chronic effect concentration, an empirical acute-to-chronic ratio (ACR) is applied (Di Toro et al., 2000; McGrath & Di Toro, 2009; McGrath et al., 2004). By integration of an empirical ACR, other mechanisms for chronic or sublethal toxicity, besides narcosis, are addressed. The TLM was adopted by the US Environmental Protection Agency (USEPA) as the technical foundation for developing criteria for PAHs and other NIOCs that act by a nonspecific mode of action such as narcosis (Burgess et al., 2008).

The initial development of the TLM, the log (LC50)–log (K_{OW}) relationship was evaluated for 33 aquatic species and more than 140 narcotic compounds (Di Toro et al., 2000). Since its original development, the TLM database has been expanded to contain 228 chemicals covering 79 species that are representative of eight taxonomic groups including fish, insects, crustaceans, algae, and higher plants (McGrath et al., 2018).

The general form of the relationship is:

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

where m and b are the slope and intercept of the regression line relating log (LC50) to log (K_{OW}), and the LC50 has units of mmol/L. It was found that a single slope describes the relationship for all species, whereas the y-intercept varies for each species (Di Toro et al., 2000). Di Toro et al. (2000) rationalized that the slope describes the relationship between the target lipid and octanol, which should be constant across species, and denoted the slope as the universal narcosis slope.

Furthermore at the y-intercept, where log (K_{OW}) = 0 (and therefore $K_{OW} = 1$), the concentration of the chemical in the octanol is equal to the concentration of the chemical in the exposure water. Assuming that octanol is a good surrogate for organism lipid, then the concentration of the chemical in water, at the y-intercept, is equal to the concentration of the chemical in the organism lipid. The y-intercept was defined as the critical target lipid body burden (CTLBB), C_L^*, with units of μmol/$g_{octanol}$. If the water-only toxicity data were LC50, then the y-intercept is equivalent to the chemical concentration in the organism lipid that will cause 50% mortality of the test species (i.e., LC50 body burden).

The TLM equation that predicts the critical aqueous concentration is:

$$\log(C_W^*) = m \log(K_{OW}) + \log(C_L^*) + \Delta c \quad (2)$$

where C_w^* is the critical aqueous concentration (mmol/L; i.e., LC50 for a mortality endpoint), C_L^* is the CTLBB (μmol/$g_{octanol}$), and Δc ($\log_{10}$ mmol/L) is a correction that is needed for some chemical classes (e.g., aromatic hydrocarbons) to account for class-specific differences in partitioning behavior to the target lipid (Kipka & Di Toro, 2009). This approach is consistent with scientific opinion that the difference between polar and nonpolar narcotics is mainly due to the differential partitioning of these molecules and not a difference in the general toxic mode of action (Kipka & Di Toro, 2009; Roberts & Costello, 2003; Vaes et al., 1998). This is also true for some ionizable compounds (Escher et al., 2020; Redman et al., 2018). The term "$m \log (K_{OW}) + \Delta c$" represents the target lipid–water partition coefficient.

MATERIALS AND METHODS

The application of the TLM to HACs took account of the availability and quality of toxicity data, development of chemical class correction factors, and evaluation of its protectiveness of chronic effects.

Toxicity data compilation and assessment of quality

Aqueous acute and chronic toxicological information for HACs was reviewed from various resources including literature searches with ProQuest Dialog, and on-line databases and data warehouses including USEPA ECOTOX and Organisation for Economic Co-operation and Development (OECD) QSAR Toolbox. The compiled acute and chronic toxicity data are for single-compound exposures using HACs. Even though O-substituted species are not expected to occur in refined petroleum substances (Comber et al., 2016), they were included for completeness. The acute endpoints evaluated included mortality for most species, immobilization for daphnids, growth rate inhibition for algae, and inhibition of feeding rate for mussels. For chronic effects and depending on taxa, endpoints of mortality, growth, and reproduction were considered.

Exposures that included UV exposure were not included in the TLM analysis to avoid the cofounding influence of UV on toxicity. Toxicity data were screened to ensure each study met specific criteria for quality and reliability. Toxicity data reliability were assessed using the fundamentals of the scoring system approach for evaluating the quality of toxicity data of Klimisch et al. (1997) and the OECD guidance of the calculation of toxicity test endpoints (OECD, 2000). These same data quality guidelines have been adopted for derivation of environmental risk limits by Dutch risk assessors (Van Vlaardingen & Verbruggen, 2007) and used in guidance documentation under the REACH regulation (European Chemicals Agency [ECHA], 2008). Each toxicity data point was assigned a ranking value from 1 to 4. Data points that received a ranking value of 1 or 2 were obtained using acceptable test protocols, had analytical confirmation of exposure concentrations, exhibited a monotonic concentration–response relationship, and had concentrations below solubility levels (e.g., bioavailable). Data points that received a ranking value of 3 had no analytical confirmation of exposure concentrations, or exhibited an inconsistent concentration–response, or the concentrations were above solubility limits. Data points received a ranking value of 4 if insufficient information was provided about exposure concentrations or test method. Data that received a ranking of 1 or 2 were used in this analysis. Data that received a ranking of 3 were deemed unreliable. A ranking value of 4 indicated the data quality was unassignable. McGrath et al. (2018) present more details on the data assessment approach used to evaluate toxicity data for the TLM framework.

Determination of TLM coefficients

Chemical class correction factors (Δc, Equation 2) were determined for HACs. These correction factors were determined using toxicity data for seven species that had three or more toxicity data points in the toxicity database compiled in the present study and had species-specific CTLBBs available in McGrath et al. (2018). Species used in the determination of the coefficients were *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, *Pimephales promelas*, *Danio rerio*, *Pseudokirchneriella subcapitata*, and *Chironomus riparius*. A two-step procedure was used to determine the chemical class coefficients. The first step was to compute a theoretical baseline LC50 (e.g., $\Delta c = 0$) value for each HAC using Equation (2), with values for the chemical's log K_{OW} (see the Supporting Information, Table S1), the species-specific CTLBBs, and the universal narcosis slope of 0.94 (McGrath et al., 2018). This computed LC50 represents the toxicity for a baseline narcotic chemical, a chemical that does not require a chemical class correction factor such as aliphatic hydrocarbons or alcohols (Di Toro et al., 2000). The second step was to determine the chemical class coefficient needed in Equation (2) to adjust the baseline LC50 to best match the observed LC50 of the HAC. In the second step, monocyclic and polycyclic structural classes were considered separately to be consistent with previous approaches. The least squares function in the R (Ver 3.6.3.) statistical programming language was used for this analysis (R Development Core Team, 2014). These HAC chemical class correction factors were then used to compute the CTLBB for species in the heterocyclic toxicity database that were not in the 2018 TLM database.

RESULTS AND DISCUSSION

Physicochemical properties of HACs

Toxicity data were available for 63 HACs. There were 33 heterocyclic compounds containing N in the aromatic ring, mostly comprised of substituted pyridines, quinolines, and acridines, 16 heterocylic compounds containing O in the aromatic ring, mostly substituted furans, and 14 compounds containing S in the aromatic ring, with the majority of chemicals being substituted thiophenes and benzothiophenes. Examples of the structural families for the HACs for which toxicity data were compiled are shown in Figure 1. A listing of all chemicals and their physical–chemical properties can be found in the Supporting Information, Table S1. Log K_{OW} and water solubility were computed using Estimation Programs Interface (EPI) Suite Ver 4.11 (USEPA, 2012). The dissociation constant (pK_a) values were calculated with the OASIS electric model and the OASIS regression model in the QSAR Toolbox Ver 4.4. The terms "electric model" and "regression model" refer to the approach taken for the calculation of the pK_a. The electric model is based on a system of equations that assumes that an atom behaves in a way similar to a circuit, and considers the potential of each atom in the structure to release/accept hydrogen atoms to calculate an overall pK_a. The regression model is a linear free energy relation (LFER) based on a group contribution approach. Both models are freely available in the QSAR Toolbox Ver 4.4.

The log K_{OW} values for the heterocyclic chemicals predicted by the KOWWIN model, the EPI Suite module for

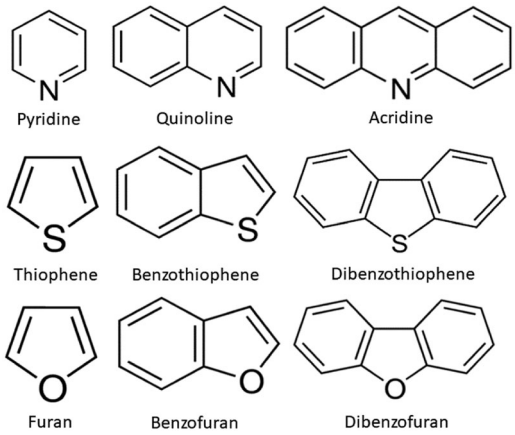

FIGURE 1: Examples of the heterocyclic aromatic compounds in the target lipid model.

log K_{OW} calculation, were deemed reliable for use in the present study. The log K_{OW} values of all compounds were within the range allowed by the model, and all the fragments in the molecules were covered in the training set of the model (including the aromatic O, N, and S moieties). No molecule had more occurrence of a single fragment than those allowed by the model, so all the chemicals analyzed were considered within the domain of KOWWIN. Futhermore, a comparison of experimental log K_{OW} values for 46 heterocyclics available in the EPI Suite Ver 4.11 database and the World Health Organization's EPI-WIN–predicted log K_{OW} values demonstrated good agreement (see the Supporting Information, Figure S1).

The calculated values of pK_a for the HACs suggest that they are not highly ionizable at environmentally relevant pH values (i.e., 5–9), supporting the assumption that these compounds behave like neutral compounds and the parent compound should be the chemical form present in solution.

Acute toxicity database

The acute toxicity data points reviewed for reliability and consideration in the derivation of the TLM chemical class coefficients for HACs are provided in the Supporting Information, Table S2. In total, 212 individual data points were reviewed, and 137 data points were assigned a ranking value of 1 or 2 (i.e., deemed reliable and accepted for further analysis). The reliable acute toxicity data represented 31 species including fish, crustaceans, mollusks, algae, and one higher plant species (Table 1). The species with the greatest number of data points were the microalgae *D. subspicatus*, the cladoceran *D. magna*, and the fish *P. promelas*, accounting for 71 data points, which combined represented 52% of the data points (i.e., 71/137). An additional 92 acute toxicity values compiled from the USEPA ECOTOX database had no confirmation of exposure concentration and, therefore, were based on nominal concentrations, which makes these values unreliable. These values were assigned a ranking of 3–4 because no other aspects of data quality were reviewed (e.g., concentration–response, test procedure). For completeness, these acute study data are summarized in the Supporting Information, Table S3.

Chronic toxicity database

There were 37 acceptable chronic endpoints for HACs from 11 species (Table 1). Chronic endpoints considered were those based on exposure durations appropriate to the species' life cycle and relevant in vivo endpoints and included the 10% effect concentration (EC10), the effect concentration (EC)/LC50, and no-observed-effect concentration (NOEC) values. The preferred statistical endpoint for risk assessment is the EC10, the concentration of the chemical predicted to produce a response to 10% of the test organisms, as opposed to the NOEC (ECHA, 2008). The NOEC has limited utility (Jager, 2012) because it is not based on a statistical model fit to the concentration–response relationship and depends heavily on the exposure concentrations tested (OECD, 1998). In the present study, when both EC10 and NOEC values were

TABLE 1: Species and number of reliable toxicity datapoints in the heterocyclic aromatic compound database

	Species	No. of acute values	No. of chronic values
Algae	*Chlamydomonas eugametos*	1	0
	Chlorella pyrenoidosa	1	1
	Desmodesmus subspicatus	15	4
	Dunaliella tertiolecta	6	0
	Navicula salinarum	1	0
	Nitzschia sigma	1	0
	Pseudokirchneriella subcapitata	8	6
	Scenedesmus acuminatus	4	8
	Staurastrum chaetoceras	1	0
	Staurastrum manfeldtii	1	0
Crustacean	*Artemia salina*	1	0
	Americamysis bahia	1	0
	Ceriodaphnia dubia	4	5
	Daphnia magna	37	4
	Daphnia pulex	3	0
	Diaptomus clavipes	0	1
	Gammarus minus	2	0
	Oithona davisae	1	0
	Palaemonetes pugio	1	0
Fish	*Danio rerio*	8	0
	Gasterosteus aculeatus	1	1
	Micropterus salmodies	2	0
	Oncorhynchus mykiss	0	4
	Oryzias latipes	1	0
	Pimephales promelas	19	2
	Poecilia reticula	1	0
Insects	*Chironomus riparius*	2	0
	Chironomus tentans	4	0
Mollusks	*Dreissena polymorpha*	5	1
	Lymnaea stagnalis	1	0
	Mytilus edulis	1	0
	Physa gyrina	1	0
Plants	*Lactuca sativa*	2	0
Total no. of data points		137	37
Total no. of species		31	11

provided, preference was given to the EC10 value. A summary of the chronic endpoints is provided in the Supporting Information, Table S2.

Determination of TLM coefficients

To determine TLM chemical class correction factors for HACs, three iterations were performed. First, the data set was separated into heterocyclic monocyclic aromatic compounds (MACs) and PACs to see whether the number of aromatic rings would be important to consider. For homocyclic aromatics (i.e., without the heteroatoms N, S, and O in the aromatic ring), the chemical class correction factors for MAHs and PAHs were –0.025 and –0.364, respectively (McGrath et al., 2018), which suggests that the number of aromatic rings may influence the partitioning of chemicals into lipid. With the heteroatoms, the chemical class coefficients were similar at –0.441 for heterocyclic MACs and –0.479 for heterocyclic PACs, suggesting that the number of aromatic rings is not an important factor to consider for partitioning of these compounds. Next, the chemicals were separated by the heteroatom, and chemical class corrections were determined for N-, O-, and S-containing aromatics. The chemical class corrections were similar for N- and O-containing aromatics at –0.486 and –0.493, respectively, and a bit lower for S-containing aromatics at –0.412. This result was not unexpected because the ability of a chemical to accept hydrogen bonds was determined to be the descriptor responsible for the differential paritioning of these compounds into lipids compared with other compounds such as alkanes and homocyclic PAHs (see the Supporting Information, Mechanistic Basis for Chemical Class Correction Factor). Nitrogen and oxygen have a high electronegativity and are known to be good hydrocarbon bond acceptors. In comparison, sulfur has a low electronegativity and is a weak hydrogen bond acceptor. Whether the compounds were separated by the number of aromatic rings or the heteroatom, the chemical class corrections were generally similar, ranging from –0.412 to –0.493, and one chemical class correction should be applicable to all the HACs. In the third iteration, all the HACs were combined, and a single chemical class correction was determined, –0.471, with a standard error of 0.055 (see Di Toro et al., 2000 for an explanation of standard errors). The results of the three iterations are summarized in Table 2. For each iteration, a root mean square error (RMSE) was computed to determine whether one approach was better (i.e., the smaller the RMSE, the better the approach). For the three iterations, the RMSE values were nearly identical at 0.499, 0.500, and 0.495, respectively. For simplicity, the third iteration, with the HACs treated as one group, was selected for further analysis.

Graphs of log (LC50 or EC50) versus log (K_{OW}) for the seven species used in the derivation of the TLM heterocyclic chemical class corrections are provided in Figure 2. The chemical class correction was applied to the measured data so that the toxicity would be normalized to baseline toxicity. To do this, the chemical class correction is subtracted from the log-transformed measured LC50 value, that is, log LC50 – Δc_j (see Equation 2). The LC50s representative of baseline toxicity were greater than the LC50s for chemicals that require a class correction, such as the heterocyclic MACs and PACs. As an example, the 2,4-dimethylquinoline LC50 for *D. rerio* was 0.063 mmol/L (Supporting Information, Table S2). The equivalent baseline LC50 was 0.190 mmol/L (i.e., $10^{(\log LC50-\Delta c_j)}$), which is a factor of 3.0 greater than the measured 2,4-dimethylquinoline LC50. Similar graphs before the normalization to baseline toxicity are provided in the Supporting Information, Figure S2, where the offset from measured toxicity (i.e., the datapoints) to the TLM baseline prediction (i.e., solid line) can be observed. The residuals (i.e., the difference between predicted and measured acute values) demonstrate no bias for chemicals with a log K_{OW} up to 5 (Supporting Information, Figure S3).

There were sufficient HAC data to compute the CTLBB for two algal species that were not included in the 2018 TLM database (McGrath et al., 2018). These species were the green algae *Dunaliella tertiolecta* and the microalgae *D. subspicatus*. The CTLBBs were computed using Equation (2) with the universal slope (m) value of –0.94 and the HAC coefficients. The resulting CTLBBs were 23.4 and 112 μmol/g octanol for *D. tertiolecta* and *D. subspicatus*, respectively. Graphs of log LC50 versus log K_{OW} are provided in the Supporting Information, Figure S4. These CTLBB values are within the range of CTLBB values for other algal species and aquatic species in general, which range from 9 to 327 μmol/g octanol (McGrath et al.,

TABLE 2: Heterocyclic chemical class correction factors for the target lipid model (TLM)

Iteration	Chemical class	TLM coefficient (Δc, log 10 mmol/L)
1: Chemicals identified as monocyclic or polycyclic aromatic compounds	Monocyclic aromatic compound	–0.441
	Polycyclic aromatic compound	–0.479
	log RMSE	0.499
2: Chemicals identified as containing either sulfur, nitrogen, or oxygen heteroatoms	Sulfur	–0.412
	Nitrogen	–0.486
	Oxygen	–0.493
	log RMSE	0.500
3: Chemicals treated as one single catergory of heterocyclic compounds	Heterocyclic aromatic compounds	–0.471
	log RMSE	0.495

RMSE = root mean square error

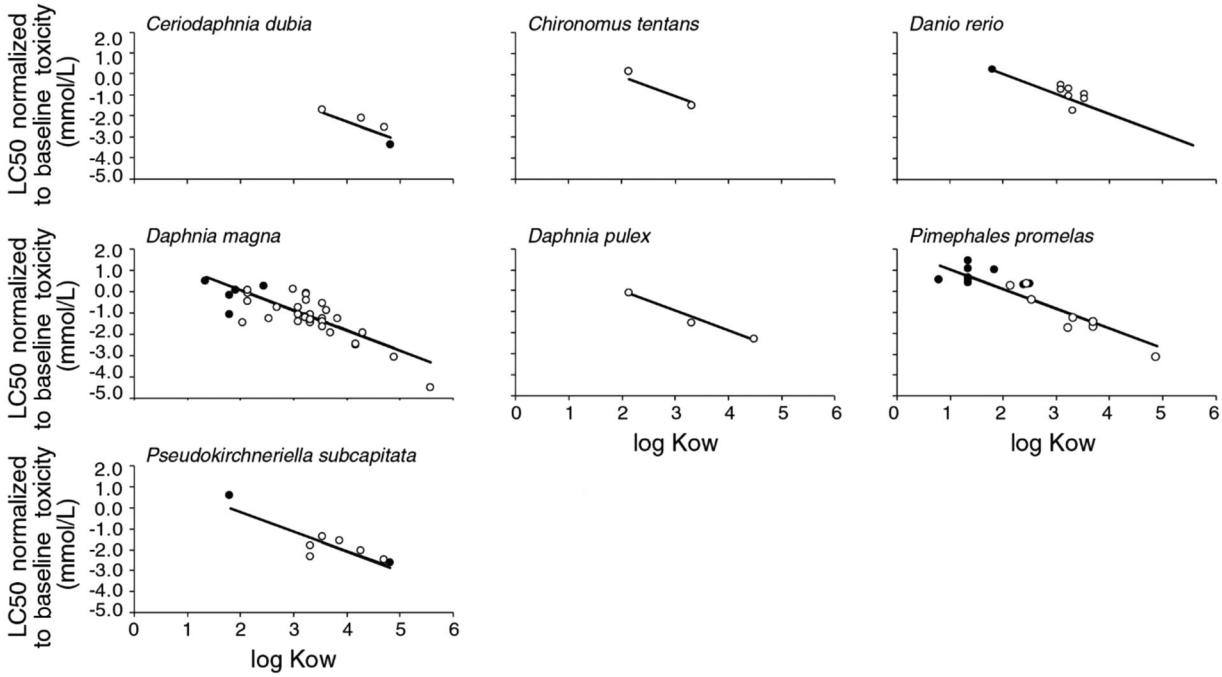

FIGURE 2: Log acute toxicity versus predicted log K_{OW} for the seven species used in the derivation of the target lipid model (TLM) heterocyclic chemical coefficients. For *Daphnia* and algal species, the acute effect is the median effect concentration (EC50). For all other species, the acute effect is the median lethal concentration (LC50). The slope is the universal narcosis slope. Toxicity was normalized to baseline. • = monocyclic aromatic compounds; O = polycyclic aromatic compounds; diagonal line = baseline TLM.

2018). McGrath et al. (2018) presented a chronic CTLBB value for *D. tertiolecta* of 7.9 µmol/g octanol. The acute and chronic endpoints for *D. tertiolecta* resulted in an ACR of 2.96, which is in the range of ACRs reported subsequently in the present study for HACs and is consistent with ACRs for MAHs and PAHs (McGrath et al., 2018).

Mechanistic basis for chemical class coefficient

The polyparameter (pp)LFER) approach provides a mechanistic basis for understanding chemical processes that control partitioning (Endo & Goss, 2014; Goss & Schwarzenbach, 2001; Redman et al., 2018) or toxicity (Boone & Di Toro, 2019; Kipka & Di Toro, 2009). For example, the class correction coefficient for heterocyclics derived in the present study (–0.471) is larger than for other chemical classes (0 baseline, –0.025 MAHs, –0.364 PAHs) calculated previously (McGrath et al., 2018). This difference is due to the more polar nature of these heterocyclic molecules. The hydrogen bond–accepting capability of the heterocyclics is noticeably greater than for homocyclic hydrocarbons. When these parameters are applied to the ppLFER model for target lipid–water partitioning (Kipka & Di Toro, 2009), it is clear that the hydrogen bonding–accepting capability is driving most of the apparent offset between the heterocyclics and other chemical classes (see the Supporting Information for details and Figures S5 and S6). The chemical class correction factor for heterocyclics is almost half a log unit lower (–0.47) than the class correction for nonpolar organics that have no polar character, such as alkanes, and approximately half of the relative lipid–water partitioning offset observed for ionic organic species (range ~10–100; Escher et al., 2020). Therefore, the empirically derived chemical class correction factors for heterocyclics appear to be consistent with mechanistic and empirical evidence.

HC5 for risk assessments

The TLM framework was used to compute a hazard concentration for 95% species protection (HC5) based on the distribution and uncertainty of the CTLBBs (C_L^*) and ACRs in the TLM database (McGrath & Di Toro, 2009; McGrath et al., 2004, 2018). The resulting equation for a chronic HC5 is:

$$\log(\mathrm{HC5}) = E[m]\log(K_{OW}) + E[\log(C_L^*)] + \Delta c - E[\log(\mathrm{ACR})] - k_Z\sqrt{\begin{array}{c}V[m]\log(K_{OW})^2 + V[\log(C_L^*)] + V[\log(\mathrm{ACR})] \\ +2\log(K_{OW})[Cov(m,\log(C_L^*)]\end{array}} \quad (3)$$

McGrath et al. (2018) presented the parameters for the HC5 equation based on the 2018 acute CTLBB and ACR databases. The universal slope was $E[m] = -0.940$ with a variance of $V[m] = 0.000225$. The log mean value of $E[\log(C_L^*)]$ was 1.85 µmol/g octanol with a variance $V[\log(C_L^*)]$ of 0.135. The log mean $E[\log(ACR)]$ and variance $V[\log(ACR)]$ values of ACR were 0.718 and 0.149, respectively. The covariance between the slope and log CTLBB $Cov(m, \log(C_L^*) = -0.0079$. The k_Z value of 2.396 was based on the smaller data set for ACRs ($n = 20$) rather than the number of CTLBBs ($n = 79$) to be conservative. The HC5 was demonstrated to be protective of chronic effects from MAHs and PAHs within the uncertainty

TABLE 3: Database of acute and chronic values used to develop the acute-to-chronic ratio

Species	Chemical	Acute LC50/ EC50 (µg/L)	Chronic endpoint	EC10 (µgL)	NOEC (µg/L)	ACR (EC10 based)	ACR (NOEC based)	Preferred ACR[a]	Genus mean ACR	Reference
Ceriodaphnia dubia	9-Ethylcarbazole	464	Reproduction	59.7	50	7.78	9.28	7.78		EMBSI, 2017
Ceriodaphnia dubia	4-Methyldibenzothiophene	174	Reproduction	29.9	35	5.82	4.97	5.82		EMBSI, 2019a
Ceriodaphnia dubia	2-Hexylthiophene	24.18	Reproduction	1.16		20.8		20.8		EMBSI, 2020a
Ceriodaphnia dubia	2-Methylthianaphthalene	926	Reproduction	337	401	2.75	2.31	2.75	7.14	EMBSI, 2020b
Chlorella pyrenoidosa	Quinoline	74000	Growth rate inhibition	63000	30000	1.17	2.47	1.17	1.17	Ramos et al., 1999
Daphnia magna	2-Methylfuran	34000	Reproduction		9000		3.78	3.78		Heger et al., 2018
Daphnia magna	Acridine	2300	Reproduction		400		5.75	5.75	4.66	Parkhurst et al., 1981
Desmodesmus subspicatus	7H-Dibenzo[*c*,*g*] carbazol	11.28	Growth rate inhibition	3.06		3.69		3.69		Brendel et al., 2018
Desmodesmus subspicatus	benzo[*b*]naphtho [1,2-d]furan	51.15	Growth rate inhibition	17.8	5.59	2.87	9.15	2.87	3.25	Brendel et al., 2018
Dreissena polymorpha	Acridine	960	Mortality		310		3.10	3.10	3.10	Kraak et al., 1997
Gasterosteus aculeatus	2,6-Dimethylquinoline	13000	Growth and mortality		1000		13.00	13.0	13.0	van den Dikkenberg et al., 1989
Pseudokirchneriella subcapitata	2-Hexylthiophene	139	Growth rate inhibition	45	14	3.09	9.93	3.09		EMBSI, 2020c
Pseudokirchneriella subcapitata	2-Methylthianaphthalene	2000	Growth rate inhibition	1000	920	2.00	2.17	2.00		EMBSI, 2020d
Pseudokirchneriella subcapitata	4-Methyldibenzothiophene	218	Growth rate inhibition	45	15	4.84	14.53	4.84		EMBSI, 2019b
Pseudokirchneriella subcapitata	9-Ethylcarbazole	566	Growth rate inhibition	354	253	1.60	2.24	1.60		EMBSI, 2018
Pseudokirchneriella subcapitata	Thiophene	113000	Growth rate inhibition	12000		9.42		9.42	3.39	OECD, 2011
Scenedesmus acuminatus	Acridine	320	Growth rate inhibition	110		2.91		2.91		van Vlaardingen et al., 1996
Scenedesmus acuminatus	Phenanthridine	5240	Growth rate inhibition	3150		1.66		1.66		van Vlaardingen et al., 1996
Scenedesmus acuminatus	Benzo[*f*]quinoline	1550	Growth rate inhibition	980		1.58		1.58		van Vlaardingen et al., 1996
Scenedesmus acuminatus	Benzo[*h*]quinoline	6650	Growth rate inhibition	4470		1.49		1.49	1.84	van Vlaardingen et al., 1996

[a]For calculation of the ACR, the EC10 is preferred over the NOEC except when the EC10 is not an absolute value.
LC50 = median lethal concentration; EC50 = median effect concentration; NOEC = no-observed-effect concentration; ACR = acute-to-chronic ratio; EC10 = 10% effect concentration.

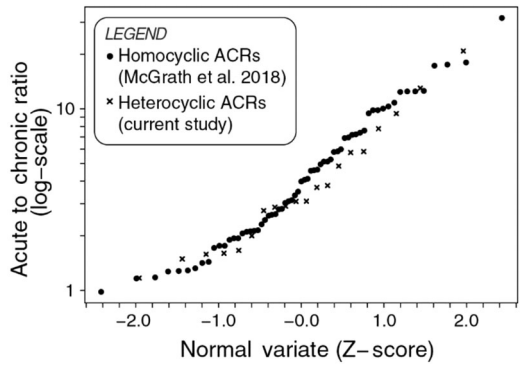

FIGURE 3: Acute-to-chronic ratio (ACR) distributions for homocyclic (McGrath et al., 2018) and heterocyclic aromatic compounds.

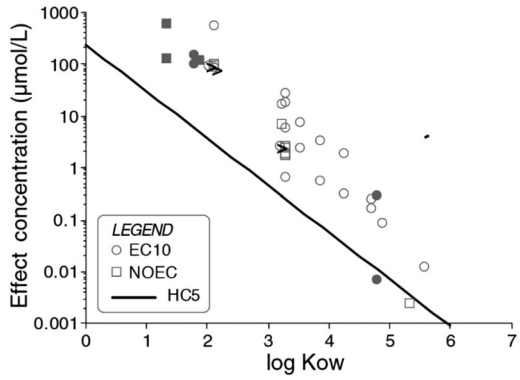

FIGURE 4: Chronic toxicity values for heterocylic monoaromatic (filled symbols) and polyaromatic (open symbols) compounds. Solid line is the concentration above which 95% of the species (HC5) should be protected for heterocylic polyaromatic compounds computed in the present study. EC10 = 10% effect concentration; NOEC = no-observed-effect concentration.

limits of the model (McGrath et al., 2018). The HC5 is a conservative threshold that is used for general purpose risk assessments (Redman et al., 2014). To validate the TLM to support risk assessment of heterocyclics, it is necessary to demonstrate that HC5 is protective of the available chronic toxicity data for this class of chemicals.

Application of the HC5 to HACs

To apply the HC5 equation to the HAC using the ACR parameters determined from the 2018 TLM database, the ACRs from the HAC database should be statistically similar to the ACRs from the 2018 TLM database. The database of HAC ACRs was compiled from reliable acute and chronic toxicity data that were generated for a given chemical and species in the same laboratory. A summary of HAC ACRs is provided in Table 3.

In the heterocyclic ACR data set, there are 20 individual ACRs ranging from 1.2 to 20.8 for eight species. The geometric average ACR across species is 3.66. Because the ACRs have an assumed log normal distribution (McGrath et al., 2018), the geometric average is computed. In comparison, the 2018 TLM database had 66 individual ACRs ranging from 1.0 to greater than 95.2 for 20 species, with a species geometric average of 5.22. The distributions of the two ACR data sets are similar (Figure 3), and three statistical tests (the *t*-test, Wilcoxon test, and Kolmogorov–Smirnov test; see the Supporting Information for details) confirmed no signficant differences between the two ACR data sets.

To determine whether the HC5 is appropriately protective of aquatic life, chronic toxicity data for HACs are compared with the HC5 (Figure 4). The solid line in Figure 4 is the HC5 for heterocyclic aromatics. There are 37 chronic toxicity data points deemed reliable that can be compared with the HC5 (Supporting Information, Table S2), with two observations falling below the HC5 line by less than a factor of 2. One data point was a *C. dubia* reproduction EC10 value of 1.16 µg/L (0.007 µmol/L) for 2-hexylthiophene (ExxonMobil Biomedical Sciences [EMBSI], 2020a). The other data point was a *D. subspicatus* growth rate NOEC value of 0.566 µg/L (0.002 µmol/L) for benzo[*b*]naphtha [2,1-d]thiophene (Brendal et al., 2018). The excursion of 2 out of 37 data points below the HC5 is consistent with the 95% protection level goal (i.e., 94.6% [35/37]). This result confirms that the TLM-derived HC5 is protective of the measured chronic effects of growth, reproduction, and mortality. Therefore, the HC5 can be used as a benchmark for assessing risk to ecological receptors from exposure to HACs.

CONCLUSIONS

Although HACs are present in petroleum substances, modeling frameworks used to assess potential risk to aquatic organisms from exposure to petroleum substances has not explicity accounted for the presence of these chemicals. Using reliable acute and chronic toxicity data, the TLM framework was demonstrated to be applicable to HACs, and the ACRs were similar to other narcotic chemicals. Chemical class coefficients for HACs were derived from available toxicity data for use in the TLM. The chemical class coefficients were larger than chemical class coefficients for other chemicals previously derived (i.e., ketones, MAHs and PAHs that do not contain heteroatoms), suggesting that the heteroatom plays a large role in the partitioning of the chemical into the organism's lipid. The TLM-derived HC5 values for heterocyclic aromatics were derived and demonstrated to be appropriately protective of chronic effects resulting from exposure to low levels of these compounds over an organism's life cycle. Given the HC5 values derived for aquatic life protection, use of equilibrium partitioning theory would allow subsequent derivation of coherent soil and sediment quality objectives. A key limitation is the potential modulating role of phototoxicity on risk assessment of this compound class, which has not been addressed. Additional site-specific PAC-UV exposure considerations coupled with recent extensions of the TLM to phototoxicity would be required to evaluate this concern. By incorporating HACs into a TLM framework, a better understanding of the potential risk from exposure to these components in petroleum substances, other substances, or environmental samples containing these

compounds can be achieved and, if warranted, subsequently managed.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5194.

Acknowledgment—The authors thank Concawe, Brussels, Belgium, for funding the present study and members of the technical committee for helpful comments during manuscript preparation.

Author Contributions Statement—J. McGrath: Review of the literature; writing and preparation of the manuscript; data analysis. G. Getzinger and M. Edwards: Data analysis. A. Redman: Writing of the manuscript; data analysis. A. Martin Aparicio: Assistance with the literature search and preparation of the manuscript. E. Vaiopoulou: Identification of data gaps; conceptualization of the research.

Data Availability Statement—All the data used are provided in the tables and Supporting Information. Data, associated metadata, and calculation tools are also available from the corresponding author (eleni.vaiopoulou@concawe.eu).

REFERENCES

Anderson, J. A., Kuhl, A. J., & Anderson, A. N. (2014). Toxicity of oil and dispersed oil on juvenile mud crabs, *Rhithropanopeus harrisii*. *Bulletin of Environmental Contamination and Toxicology*, *92*, 375–380.

Bleeker, E. A. J., van der Geest, H. G., Kraak, M. H. S., de Voogt, P., & Admiraal, W. (1998). Comparative ecotoxicity of NPAHs to larvae of the midge *Chironomus riparius*. *Aquatic Toxicology*, *41*, 51–62.

Boone, K. S., & Di Toro, D. M. (2019). Target site model: Application of the polyparameter target lipid model to predict aquatic organism acute toxicity for various modes of action. *Environmental Toxicology and Chemistry*, *38*, 222–239.

Brendel, S., Polleichtner, C., Behnke, A., Jessel, S., Hassold, E., Jennemann, C., Einhenkel-Arle, D., & Seidel, A. (2018). Four selected high molecular weight heterocyclic aromatic hydrocarbons: Ecotoxicological hazard assessment, environmental relevance and regulatory needs under REACH. *Ecotoxicology and Environmental Safety*, *163*, 340–348.

Burgess, R. M., Berry, W. J., Mount, D. R., Ankley, G. T., Ireland, S., Di Toro, D. M., Hansen, D. J., McGrath, J. A., DeRosa, L. D., Bell, H., Keating, J. F., Reiley, M. C., & Zarba, C. S. (2008). Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: Compendium of tier 2 values for nonionic organics (EPA/600/R-02/016). Technical Report. US Environmental Protection Agency.

Comber, M. H. I., de Ferrer, J. A., Djemel, N., Eadsforth, C. V., Lescrauwaet, A., Leon Paumen, M., Linington, S., Redman, A., & Villalobos, S. A. (2016). Analysis of N-, O-, and S-heterocyclics in petroleym products using GCxGC with specific detection (Concawe Report 2/16). Concawe.

Concawe. (n.d.). PetroTox and PetroRisk. https://www.concawe.eu

Di Toro, D. M., McGrath, J. A., & Hansen, D. J. (2000). Technical basis for narcotic chemicals and PAH criteria. I. Water and tissue. *Environmental Toxicology and Chemistry*, *19*, 1951–1970.

Dijkman, N. A., van Vlaardingen, L. A., & Admiraal, W. A. (1997). Biological variation in sensitivity to N-heterocyclic PAHs; effects of acridine on seven species of micro-algae. *Environmental Pollution*, *95*, 121–126.

Endo, S., & Goss, K. U. (2014). Applications of polyparameter linear free energy relationships in environmental chemistry. *Environmental Science & Technology*, *48*, 12477–12491.

Escher, B. I., Abagyan, R., Embry, M., Kluver, N., Redman, A. D., Zarfi, C., & Parkerton, T. F. (2020). Recommendations for approving methods and models for aquatic hazard assessment of ionizable organic chemicals. *Environmental Toxicology and Chemistry*, *39*, 269–286.

European Commission. (2006). Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). *Official J Eur Union*, *L396*, 1–849.

European Chemicals Agency. (2008). Characterization of dose [concentration]-response for environment. In *Guidance on information requirements and chemical safety assessment*.

ExxonMobil Biomedical Sciences. (2017). *Ceriodaphnia dubia* survival and reproduction test for 9-ethylcarbazole (Study Number 1713496).

ExxonMobil Biomedical Sciences. (2018). Algal growth inhibition test for 9-ethylcarbazole (Study Number 1713467).

ExxonMobil Biomedical Sciences. (2019a). *Ceriodaphnia dubia* survival and reproduction test for 4-methyldibenzothiophene (Study Number 1863796).

ExxonMobil Biomedical Sciences. (2019b). Algal growth inhibition test for 4-methyldibenzothiophene (Study Number 1863767).

ExxonMobil Biomedical Sciences. (2020a). *Ceriodaphnia dubia* survival and reproduction test for 2-hexylthiophene (Study Number 1917296).

ExxonMobil Biomedical Sciences. (2020b). *Ceriodaphnia dubia* survival and reproduction test for 2-methylthianaphthalene (Study Number 1885496).

ExxonMobil Biomedical Sciences. (2020c). Algal growth inhibition test for 2-hexylthiophene (Study Number 1917267).

ExxonMobil Biomedical Sciences. (2020d). Algal growth inhibition test for 2-methylthianaphthalene (Study Number 1885467).

Goss, K. U., & Schwarzenbach, R. P. (2001). Linear free energy relationships used to evaluate equilibrium partitioning of organic chemicals. *Environmental Science & Technology*, *35*, 1–9.

Harman, C., Thomas, K. V., Tollefsen, K. E., Meier, S., Bøyum, O., & Grung, M. (2009). Monitoring the freely dissolved concentrations of polycyclic aromatic hydrocarbons (PAH) and alkylphenols (AP) around a Norwegian oil platform by holistic passive sampling. *Marine Pollution Bulletin*, *58*, 1671–1679.

Heger, S., Bluhm, K., Du, M., Lehmann, G., Anders, N., Dechambre, D., Bardow, A., Schäffer, A., & Hollert, H. (2018). Aquatic toxicity of biofuel candidates on *Daphnia magna*. *Ecotoxicology and Environmental Safety*, *164*, 125–130.

Hodson, P. V., Wallace, S. J., de Solla, S. R., Head, S. J., Hepditch, S. L. J., Parrott, J. L., Thomas, P. J., Berthiaume, A., & Langlois, V. S. (2020). Polycyclic aromatic compounds (PACs) in the Canadian environment: The challenges of ecological risk assessments. *Environmental Pollution*, *266*, 115165.

Jager, T. (2012). Bad habits die hard: The NOEC's persistence reflects poorly on ecotoxicology. *Environmental Toxicology and Chemistry*, *31*, 228–229.

King, D. J., Lyne, R. L., Girling, A., Peterson, D. R., Stephenson, R., & Short, D. (1996). Environmental risk assessment of petroleum substances: The hydrocarbon block method (Concawe Report 96/52). Concawe.

Kipka, U., & Di Toro, D. M. (2009). Technical basis for polar and nonpolar narcotic chemicals and polycyclic aromatic hydrocarbon criteria. III. A polyparameter model for target lipid partitioning. *Environmental Toxicology and Chemistry*, *28*, 1429–1438.

Klimisch, H. J., Andreae, M., & Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory and Toxicologic Pharmacology*, *25*, 1–5.

Kraak, M. H. S., Ainscough, C., Fernandez, A., van Vlaardingen, P. L. A., deVoogt, P., & Admiraal, A. (1997). Short-term and chronic exposure of the zebra mussel (*Dreissena polymorpha*) to acridine: Effects and metabolism. *Aquatic Toxicology*, *37*, 9–20.

McCarty, L. S., Mackay, D., Smith, A. D., Ozburn, G. W., & Dixon, D. G. (1991). Interpreting aquatic toxicity QSARs: The significance of toxicant body residues at the pharmacologic endpoint. *Science of the Total Environment*, *109–110*, 515–525.

McGrath, J. A., & Di Toro, D. M. (2009). Validation of the target lipid model for toxicity assessment of residual petroleum constituents: Monocyclic and polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry*, *28*, 1130–1148.

McGrath, J. A., Parkerton, T. F., & Di Toro, D. M. (2004). Application of the narcosis target lipid model to algal toxicity and deriving

predicted-no-effect concentrations. *Environmental Toxicology and Chemistry*, *23*, 2503–2517.

McGrath, J. A., Parkerton, T., Hellweger, F., & Di Toro, D. M. (2005). Validation of the narcosis target lipid model for petroleum products: Gasoline as a case study. *Environmental Toxicology and Chemistry*, *24*, 2382–2394.

McGrath, J. A., Fanelli, C., Di Toro, D. M., Parkerton, T. F., Redman, A. D., Paumen, M. L., Comber, M., Eadsforth, C. V., & den Haan, K. (2018). Re-evaluation of target lipid model-derived HC5 predictions for hydrocarbons. *Environmental Toxicology and Chemistry*, *37*, 1579–1593.

O'Shaughnessy, K. A., Forth, H., Takeshita, R., & Chesney, E. J. (2018). Toxicity of weathered Deepwater Horizon oil to bay anchovy (*Anchoa mitchilli*). *Ecotoxicology and Environmental Safety*, *148*, 473–479.

Organisation for Economic Co-operation and Development. (1998). Report of the OECD workshop on statistical analysis of aquatic toxicity data. *OECD series on testing and assessment Number 10* (ENV/MC/CHEM (98)18).

Organisation for Economic Co-operation and Development. (2000). Guidance document on aquatic toxicity testing of difficult substances and mixtures. *OECD series on testing and assessment Number 23* (ENV/JM/MONO(2000)6).

Organisation for Economic Co-operation and Development. (2011). *SIDS initial assessment report for CoCAM1*. Paris, France 10–12 October 2011.

Parkhurst, B. R., Bradshaw, A. S., Forte, J. L., & Wright, G. P. (1981). The chronic toxicity to *Daphnia magna* of acridine, a representative azaarene present in synthetic fossil fuel products and wastewaters. *Environmental Pollution*, *24*, 21–30.

Peddinghaus, S., Brinkmann, M., Bluhm, K., Sagner, A., Hinger, G., Braunbeck, T., Eisentrager, A., Tiehm, A., Hollert, H., & Keiter, S. H. (2012). Quantitative assessment of the embryotoxic potential of NSO-heterocyclic compounds using zebrafish (*Danio rerio*). *Reproductive Toxicology*, *33*, 224–232.

Quann, R. J. (1998). Modeling the chemistry of complex petroleum mixtures. *Environmental Health Perspectives*, *106*, 1441–1448.

R Development Core Team. (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.R-project.org/.

Ramos, E. U., Vaes, W. H. J., Mayer, P., & Hermens, J. L. M. (1999). Algal growth inhibition of *Chlorella pyrenoidosa* by polar narcotic pollutants: Toxic cell concentrations and QSAR modeling. *Aquatic Toxicology*, *46*, 1–10.

Redman, A. D., Parkerton, T. F., McGrath, J. A., & Di Toro, D. M. (2012). PETROTOX: An aquatic toxicity model for petroleum substances. *Environmental Toxicology and Chemistry*, *31*, 2498–2506.

Redman, A. D., Parkerton, T. F., Comber, M. H., Paumen, M. L., Eadsforth, C. V., Dmytrasz, B., King, D., Warren, C. S., den Hann, K., & Djemel, N. (2014). PETRORISK: A risk assessment framework for petroleum substances. *Integrated environmental assessment and management*, *10*, 437–448.

Redman, A. D., & Parkerton, T. F. (2015). Guidance for improving comparability and relevance of oil toxicity tests. *Marine Pollution Bulletin*, *98*, 156–170.

Redman, A. D., Parkerton, T. F., Butler, J. D., Letinski, D. J., Frank, R. A., Hewitt, L. M., Bartlett, A. J., Gillis, P. L., Marentette, J. R., Parrot, J. L., Hughes, S. A., Guest, R., Bekele, A., Zhang, K., Morandi, G., Wiseman, S., & Giesey, J. P. (2018). Application of the target lipid model and passive samplers to characterize the toxicity of bioavailable organics in oil sands process-affected water. *Environmental Science & Technology*, *52*, 8039–8049.

Roberts, D. W., & Costello, J. F. (2003). Mechanisms of action for general and polar narcosis: A difference in dimension. *Molecular Informatics*, *22*, 226–233.

Schwarz, M., Behnke, A., Brandt, M., Eisentrager, A., Hassauer, M., Kalberlah, F., & Seidel, A. (2014). Semipolar aromatic compounds: Identification of 15 priority substances and the need for regulatory steps under REACH regulation. *Integrated environmental assessment and management*, *10*, 415–428.

Serrano, B., Verhaegen, Y., den Haan, K., Camenzuli, L., Saunders, D., & Aparico, A. M. (2020). PetroTox version 4.0 user manual (Concawe Report no. 7.20). Concawe.

U.S. Environmental Protection Agency. (2012). Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11.

Vaes, W. H. J., Ramos, E. U., Verhaar, H. J. M., & Hermens, J. L. M. (1998). Acute toxicity of nonpolar versus polar narcosis: Is there a difference? *Environmental Toxicology and Chemistry*, *17*, 1380–1384.

van den Dikkenberg, R., Canton, H. H., Mathijssen-Spiekman, L. A. M., & Roghair, C. J. (1989). The usefulness of *Gasterosteus aculeatus*, the three-spined sickleback, as a test organism in routine toxicity tests (Report Number. 718625003). National Institute for Public Health and the Environment.

Van Vlaardingen, P. L. A., Steinhoff, W. J., de Voogt, P., & Admiraal, W. A. (1996). Property-toxicity relationships of azaarenes to the green alga *Scenedesmus acuminatus*. *Environmental Toxicology and Chemistry*, *15*, 2035–2042.

Van Vlaardingen, P. L. A., & Verbruggen, E. M. J. (2007). Guidance for the derivation of environmental risk limits within the framework of 'International and national environmental quality standards for substances in the Netherlands' (INS; Revision 2007; RIVM report 601782001). National Institute for Public Health and the Environment.

van Wezel, A. P., & Opperhuizen, A. (1995). Narcosis due to environmental pollutants in aquatic organisms: Residue-based toxicity, mechanisms, and membrane Bbrdens. *Critical Reviews in Toxicology*, *25*, 255–279.

Veith, G., Call, D. J., & Brooke, L. (1983). Structure–toxicity relationships for the fathead minnow, *Pimephales promelas*: Narcotic industrial chemicals. *Canadian Journal of Fisheries and Aquatic Science*, *40*, 743–748.

