

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

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**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

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[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 environment 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  $\log(\text{LC50})$ – $\log(K_{OW})$  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  $\log(\text{LC50})$  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  $\mu\text{mol}/\text{g}_{\text{octanol}}$ . 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 ( $\mu\text{mol}/\text{g}_{\text{octanol}}$ ), and  $\Delta c$  ( $\log_{10}$  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 non-polar 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 confounding 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 ( $\Delta c$ , Equation 2) were determined for HACs. These correction factors were determined

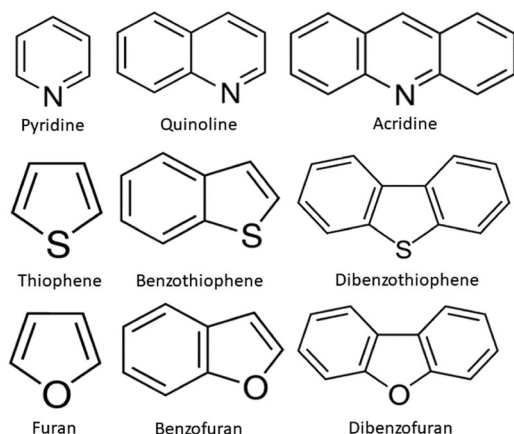
using toxicity data for seven species that had three or more toxicity data points in the toxicity database compiled in the present study and had species-specific CTLBBs available in McGrath et al. (2018). Species used in the determination of the coefficients were *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia 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 heterocyclic 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. Furthermore, 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	<i>Chlamydomonas eugametos</i>	1	0	
	<i>Chlorella pyrenoidosa</i>	1	1	
	<i>Desmodesmus subspicatus</i>	15	4	
	<i>Dunaliella tertiolecta</i>	6	0	
	<i>Navicula salinarum</i>	1	0	
	<i>Nitzschia sigma</i>	1	0	
	<i>Pseudokirchneriella subcapitata</i>	8	6	
	<i>Scenedesmus acuminatus</i>	4	8	
	<i>Staurastrum chaetoceras</i>	1	0	
	<i>Staurastrum manfeldtii</i>	1	0	
	Crustacean	<i>Artemia salina</i>	1	0
		<i>Americamysis bahia</i>	1	0
		<i>Ceriodaphnia dubia</i>	4	5
<i>Daphnia magna</i>		37	4	
<i>Daphnia pulex</i>		3	0	
<i>Diaptomus clavipes</i>		0	1	
<i>Gammarus minus</i>		2	0	
<i>Oithona davisae</i>		1	0	
<i>Palaemonetes pugio</i>		1	0	
Fish		<i>Danio rerio</i>	8	0
	<i>Gasterosteus aculeatus</i>	1	1	
	<i>Micropterus salmoides</i>	2	0	
	<i>Oncorhynchus mykiss</i>	0	4	
	<i>Oryzias latipes</i>	1	0	
	<i>Pimephales promelas</i>	19	2	
	<i>Poecilia reticula</i>	1	0	
	Insects	<i>Chironomus riparius</i>	2	0
		<i>Chironomus tentans</i>	4	0
Mollusks	<i>Dreissena polymorpha</i>	5	1	
	<i>Lymnaea stagnalis</i>	1	0	
	<i>Mytilus edulis</i>	1	0	
Plants	<i>Physa gyrina</i>	1	0	
	<i>Lactuca sativa</i>	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 partitioning 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.

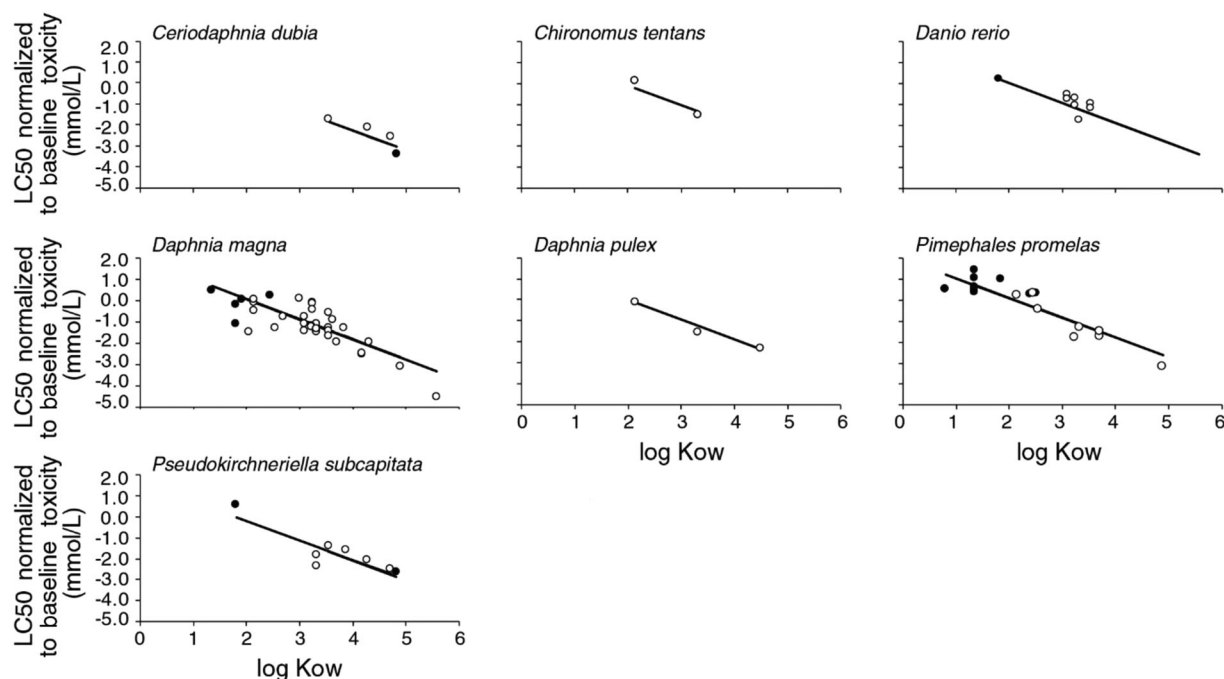
Graphs of  $\log(\text{LC50 or EC50})$  versus  $\log(K_{OW})$  for the seven species used in the derivation of the TLM heterocyclic chemical class corrections are provided in Figure 2. The chemical class correction was applied to the measured data so that the toxicity would be normalized to baseline toxicity. To do this, the chemical class correction is subtracted from the log-transformed measured LC50 value, that is,  $\log \text{LC50} - \Delta c_j$  (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.,  $10^{(\log \text{LC50} - \Delta c_j)}$ ), 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  $\mu\text{mol/g}$  octanol for *D. tertiolecta* and *D. subspicatus*, respectively. Graphs of  $\log \text{LC50}$  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  $\mu\text{mol/g}$  octanol (McGrath et al.,

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

Iteration	Chemical class	TLM coefficient ( $\Delta c$ , $\log_{10}$ 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
	Heterocyclic aromatic compounds	-0.471
3: Chemicals treated as one single category of heterocyclic compounds	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; ○ = polycyclic aromatic compounds; diagonal line = baseline TLM.

2018). McGrath et al. (2018) presented a chronic CTLBB value for *D. tertiolecta* of 7.9  $\mu\text{mol/g}$  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:

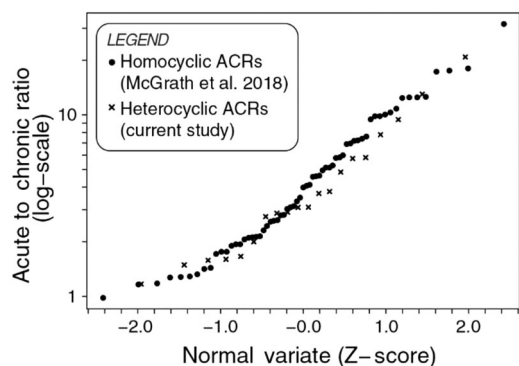
$$\log(\text{HC5}) = E[m] \log(K_{OW}) + E[\log(C_L^*)] + \Delta c - E[\log(\text{ACR})] - k_z \sqrt{\frac{V[m] \log(K_{OW})^2 + V[\log(C_L^*)] + V[\log(\text{ACR})]}{+2 \log(K_{OW}) [\text{Cov}(m, \log(C_L^*))]}} \quad (3)$$

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  $\mu\text{mol/g}$  octanol with a variance  $V[\log(C_L^*)]$  of 0.135. The log mean  $E[\log(\text{ACR})]$  and variance  $V[\log(\text{ACR})]$  values of ACR were 0.718 and 0.149, respectively. The covariance between the slope and log CTLBB  $\text{Cov}(m, \log(C_L^*)) = -0.0079$ . 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 (µg/L)	NOEC (µg/L)	ACR (EC10 based)	ACR (NOEC based)	Preferred ACR <sup>a</sup>	Genus mean ACR	Reference
<i>Ceriodaphnia dubia</i>	9-Ethylcarbazole	464	Reproduction	59.7	50	7.78	9.28	7.78		EMBSI, 2017
<i>Ceriodaphnia dubia</i>	4-Methyldibenzothiophene	174	Reproduction	29.9	35	5.82	4.97	5.82		EMBSI, 2019a
<i>Ceriodaphnia dubia</i>	2-Hexylthiophene	24.18	Reproduction	1.16		20.8		20.8		EMBSI, 2020a
<i>Ceriodaphnia dubia</i>	2-Methylthianaphthalene	926	Reproduction	337	401	2.75	2.31	2.75	7.14	EMBSI, 2020b
<i>Chlorella pyrenoidosa</i>	Quinoline	74000	Growth rate inhibition	63000	30000	1.17	2.47	1.17	1.17	Ramos et al., 1999
<i>Daphnia magna</i>	2-Methylfuran	34000	Reproduction		9000		3.78	3.78		Heger et al., 2018
<i>Daphnia magna</i>	Acridine	2300	Reproduction		400		5.75	5.75		Parkhurst et al., 1981
<i>Desmodesmus</i> <i>subspicatus</i>	7H-Dibenzo[c,g] carbazol	11.28	Growth rate inhibition	3.06		3.69		3.69		Brendel et al., 2018
<i>Desmodesmus</i> <i>subspicatus</i>	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
<i>Dreissena polymorpha</i>	Acridine	960	Mortality		310		3.10	3.10		Kraak et al., 1997
<i>Gasterosteus aculeatus</i>	2,6-Dimethylquinoline	13000	Growth and mortality		1000		13.00	13.0		van den Dikkenberg et al., 1989
<i>Pseudokirchneriella</i> <i>subcapitata</i>	2-Hexylthiophene	139	Growth rate inhibition	45	14	3.09	9.93	3.09		EMBSI, 2020c
<i>Pseudokirchneriella</i> <i>subcapitata</i>	2-Methylthianaphthalene	2000	Growth rate inhibition	1000	920	2.00	2.17	2.00		EMBSI, 2020d
<i>Pseudokirchneriella</i> <i>subcapitata</i>	4-Methyldibenzothiophene	218	Growth rate inhibition	45	15	4.84	14.53	4.84		EMBSI, 2019b
<i>Pseudokirchneriella</i> <i>subcapitata</i>	9-Ethylcarbazole	566	Growth rate inhibition	354	253	1.60	2.24	1.60		EMBSI, 2018
<i>Pseudokirchneriella</i> <i>subcapitata</i>	Thiophene	113000	Growth rate inhibition	12000		9.42		9.42	3.39	OECD, 2011
<i>Scenedesmus acuminatus</i>	Acridine	320	Growth rate inhibition	110		2.91		2.91		van Vlaardingen et al., 1996
<i>Scenedesmus acuminatus</i>	Phenanthridine	5240	Growth rate inhibition	3150		1.66		1.66		van Vlaardingen et al., 1996
<i>Scenedesmus acuminatus</i>	Benzo[f]quinoline	1550	Growth rate inhibition	980		1.58		1.58		van Vlaardingen et al., 1996
<i>Scenedesmus acuminatus</i>	Benzo[h]quinoline	6650	Growth rate inhibition	4470		1.49		1.49	1.84	van Vlaardingen et al., 1996

<sup>a</sup>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.

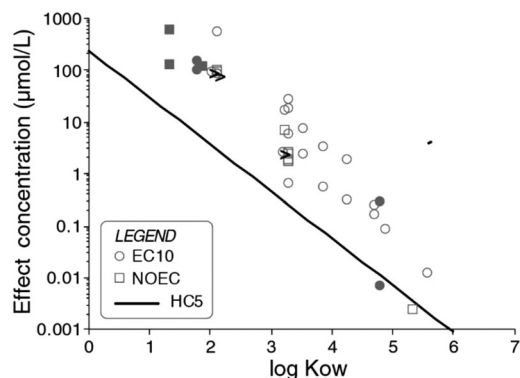
limits of the model (McGrath et al., 2018). The HC5 is a conservative threshold that is used for general purpose risk assessments (Redman et al., 2014). To validate the TLM to support risk assessment of heterocyclics, it is necessary to demonstrate that HC5 is protective of the available chronic toxicity 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 significant 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  $\mu\text{g/L}$  (0.007  $\mu\text{mol/L}$ ) for 2-hexylthiophene (ExxonMobil Biomedical Sciences [EMBSI], 2020a). The other data point was a *D. subspicatus* growth rate NOEC value of 0.566  $\mu\text{g/L}$  (0.002  $\mu\text{mol/L}$ ) for benzo[*b*]naphtha [2,1-*d*]thiophene (Brendal et al., 2018).



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

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 explicitly 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>.

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**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](mailto:eleni.vaiopoulou@concawe.eu)).

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