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VALIDATION OF THE TARGET LIPID MODEL FOR TOXICITY ASSESSMENT OF RESIDUAL PETROLEUM CONSTITUENTS: MONOCYCLIC AND POLYCYCLIC AROMATIC HYDROCARBONS

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Abstract—A method is presented for developing scientifically defensible, numeric guidelines for residual petroleum-related constituents, specifically monocyclic aromatic hydrocarbons (MAHs) and polycyclic aromatic hydrocarbons (PAHs), in the water column. The guidelines are equivalent to a HC5 (i.e., hazard concentration to 5% of the tested species, or the concentration that protects 95% of the tested species). The model of toxicity used in this evaluation is the target lipid model (TLM) that was developed for assessing the toxicity of type I narcotic chemicals. An acute to chronic ratio is used for chronic expression and sublethal effects. The TLM is evaluated by comparing predicted and observed toxicity of these petroleum components. The methodology is capable of predicting both the acute and chronic toxicity of MAHs and PAHs in single exposures and in mixtures. For acute exposures, the TLM was able to predict the toxicity to within a factor of three to five. The use of toxic units was an effective metric for expressing the toxicity of mixtures. Within the uncertainty bounds, the TLM correctly predicted where sublethal effects of edemas, hemorrhaging, and other abnormalities were observed to occur in early life-stage exposure to PAHs. The computed HC5s were lower than no-observed-effect concentrations based on growth, reproduction, and mortality endpoints and sublethal effects. The methodology presented can be used by the oil spill community to compare residual concentrations of PAHs against defensible, numeric guidelines to assess potential ecological impacts.

Keywords—Polycyclic aromatic hydrocarbons Target lipid model Model validation Petroleum toxicity Guidelines

INTRODUCTION

The toxicity of fresh petroleum often is attributed to the water-soluble monocyclic aromatic hydrocarbons (MAHs; i.e., benzene, toluene, ethylbenzene, and xylene, commonly referred to as BTEX) and the polycyclic aromatic hydrocarbons (PAHs). The low-molecular-weight BTEX compounds usually do not persist in the environment, because they are volatile compounds and are highly biodegradable. The high-molecular-weight PAHs are less volatile and degrade more slowly, and the heavier PAHs (those with four or more rings) persist and can potentially have a long-term impact on the aquatic environment. A method is presented here for evaluating the toxicity of low levels of residual petroleum based on these constituents. The model used is the target lipid model (TLM) that was derived for type I narcotic compounds, such as MAHs and PAHs. Other water-soluble compounds (e.g., phenols and heterocyclic compounds) are present in petroleum and may contribute to the toxicity; however, because the majority of the toxicity literature focuses on MAHs and PAHs, these are the primary compounds evaluated in the present paper.

The TLM and the concept of toxic units (TUs) have been used to assess the toxicity of MAHs and PAHs in water [1] and sediments [2]. The TLM was developed using toxicity data for type I narcotic chemicals in general rather than toxicity data for MAHs and PAHs specifically. The U.S. Environmental Protection Agency (EPA) developed sediment benchmarks for PAH mixtures based on the TLM [3]. These benchmarks were derived with the objective of protecting sediment organisms from long-term effects on mortality, growth, and reproduction.

Recent literature suggests that exposure to PAHs during an organism's early life stage results in various sublethal effects, such as yolk sac edemas, pericardial edemas, craniofacial malformations, and hemorrhaging [4–6]. These sublethal effects were not addressed in the original model development or in the U.S. EPA sediment benchmarks. The present study examines the degree to which the TLM can predict the toxicity of MAHs and PAHs as single chemicals and in mixtures for acute and chronic exposures. Additionally, the TLM is evaluated to determine if computed endpoints are protective of the various larval abnormalities and sublethal endpoints that are observed from early life-stage exposure to PAHs.

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MATERIALS AND METHODS

Target lipid model

Acute toxicity. The TLM [1,2] is based on the hypothesis that the aqueous concentration for a toxic endpoint, such as the median lethal concentration (LC50), can be predicted from the critical body burden in the target lipid of an organism, C_L^*, which can be calculated from the target lipid–water partition coefficient, K_{LW}:

$$C_L^* = (K_{LW})(\text{LC50}) \quad (1)$$

The superscript * denotes the concentration at which the toxic endpoint occurs. Two assumptions of the TLM are that the species-specific critical target lipid body burden (CTLBB) C_L^* is the same for any narcotic chemical and that the chemical-specific target K_{LW} is the same for any aquatic organism. The validity of these assumptions is tested implicitly by the comparisons of observed and predicted endpoints.

To apply the TLM, both C_L^* and K_{LW} are needed to predict the LC50. The target K_{LW} is obtained from a linear free-energy relationship:

$$\log(K_{LW}) = a_0 + a_1 \log(K_{OW}) \quad (2)$$

where K_{OW} is the octanol water partition coefficient of the chemical. Equations 1 and 2 are combined to produce a single linear relationship between log(LC50) and log(K_{OW}):

$$\log(\text{LC50}) = \log(C_L^*) - a_0 - a_1\log(K_{OW}) \quad \text{or} \quad (3)$$

$$\log(\text{LC50}) = m\log(K_{OW}) + b \quad (4)$$

where m and b are the slope and intercept, respectively, of the regression line relating log(LC50) to log(K_{OW}). The slope $m = -a_1$ is the slope of the linear free-energy relationship between the target lipid and octanol (Eqn. 2). Assuming the target lipid has the same chemical partitioning property in all organisms, the slope should be the same for all species. The intercept $b = -a_0 + \log(C_L^*)$ involves a parameter a_0 (Eqn. 2) and the critical target lipid concentration C_L^*. In the TLM, it is assumed that $a_0 = 0$ and that the intercept is the CTLBB. The assumption is validated by comparing the CTLBB estimated from Equation 4 and measured critical body burdens (see Fig. 10 in Di Toro et al. [1]).

The TLM has been validated extensively using a large database to predict the aquatic toxicity of type I narcotic chemicals. The CTLBBs for 33 aquatic species [1] and five algae [7] were computed using the K_{OW} values determined from a 1997 version of SPARC (Sparc Performs Automated Reasoning in Chemistry), a U.S. EPA–sponsored computer program that estimates physicochemical properties for chemicals based on chemical structure [8]. To take advantage of recent enhancements to SPARC, a 2006 version was used to determine K_{OW} values for all chemicals in the narcosis toxicity database, and revised coefficients for the TLM were computed (see Appendix 1 for coefficients).

The following TLM equation predicts the critical aqueous concentration:

$$\log(C_W^*) = m\log(K_{OW}) + \log(C_L^*) + \Delta c \quad (5)$$

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 is a chemical class correction ($\log_{10}$ mmol/L) [1]. The CTLBBs range from 24.5 to 500 μmol/g octanol. An assumption of this model is that octanol is a good surrogate for the lipid of an organism. Therefore, although the CTLBB is expressed as per gram of octanol, the CTLBB is the chemical concentration in the organism lipid needed to cause 50% mortality of the organisms. The universal slope m was determined to be −0.936. The chemical class correction Δc ranges from zero for baseline narcotics (e.g., alkanes and alcohols) to −0.109 for MAHs and −0.352 for PAHs.

The CTLBBs for nine additional species not included in the original model development have been computed from more recently available acute toxicity data. A total of 47 CTLBBs are available and are provided in Appendix 1. If the acute toxicity data for a particular species included four or more data points, the corresponding standard errors were computed. For the two species that had three data points, *Hyalella azteca* and *Chironomus tentans*, standard errors were not computed, because the three data points were all for the same compound.

Chronic toxicity. The critical aqueous concentrations, C_W^*, computed from Equation 5 are acute concentrations that produce an effect in a short-term test (i.e., 96-h LC50). To convert the acute critical concentration to a chronic effect concentration, the TLM adopts the acute to chronic ratio (ACR) methodology used by the U.S. EPA in deriving water-quality criteria [9]. The ACR is computed from paired acute and chronic toxicity data for a particular chemical to a specific organism as follows:

$$\text{ACR} = \frac{\text{acute effect concentration}}{\text{chronic effect concentration}} \quad (6)$$

where the concentrations are in the same units. The chronic effect concentrations are those that cause an adverse effect on the organism's ability to survive, grow, or reproduce on a long-term basis as appropriate for the species.

The ACR is an empirical method for estimating chronic toxicity from acute toxicity. It does not assume that the toxic mode of action is the same for acute and chronic toxicity. For the application of the TLM, the mechanism for acute effects is assumed to be narcosis. For chronic effects, the mechanisms can be diverse. In the TLM development, ACRs for 34 paired acute and chronic data sets from six different species and several type I narcotic chemicals were analyzed [1]. The ACRs ranged from 1.2 to 23, with a geometric mean of 5.09. Because the ACRs were similar for narcotic chemicals, an average ACR was appropriate. A similar approach is used below, with ACRs computed from toxicity data for MAHs and PAHs.

Computation of uncertainty. The uncertainty associated with the critical acute and chronic aqueous concentrations, C_W^*, results from the uncertainty in the variables in Equations 5 and 6. A method has been presented that adapts the HC5 methodology for computing the hazard concentration at which 5% of species are affected [7]. This method is appropriate for small sample sets and assumes that the effect concentrations conform to a log-normal or log-logistic distribution. The method has been used to compute the concentration that protects 95% of the tested species from adverse effects by considering the variance in all the available CTLBBs, C_L^* [7]. Here, the method is used to compute the 5 and 95% confidence limits (CL) for computed aqueous concentration for a particular species. The result is

$$\begin{aligned}\log C^*_{W,5\%,95\%} = {} & \log(K_{OW})E(m) + E[\log(C_L^*)] + E(\Delta c) \\ & - E[\log(\text{ACR})] \\ & \pm k_Z\{[\log(K_{OW})]^2V(m) + V(\log C_L^*) \\ & + V[\log(\text{ACR})]\}^{1/2} \end{aligned} \quad (7)$$

where $C^*_{W,5\%,95\%}$ (mmol/L) are the 5% (−) and 95% (+) CL and $\{\Delta c_i\}$ is the chemical class correction factor for chemical class i. For a specific aquatic species, if the measured water concentration is below $C^*_{W,5\%}$, we have a 95% level of confidence that adverse effects will not be observed. The $E\{\ \}$ and $V\{\ \}$ terms in Equation 7 represent the mean and variance, respectively, of the slope, C_L^*, and ACR. The k_Z is the 95% confidence sample size–dependent extrapolation factor [10] and will vary for each aquatic species depending on how many data are available. These values are provided in Appendix 1. The $C^*_{W,5\%}$ and $C^*_{W,95\%}$ values represent the lower and upper CL, respectively, of the acute and chronic concentrations. The ACR terms are only considered when computing chronic effect concentrations.

Toxicity of mixtures. The TLM approach incorporates TUs to compute the toxicity of PAH mixtures in water, tissue, and sediments [2]. The TLM and TU concept were validated for predicting the toxicity of gasoline, a complex mixture of hydrocarbons [11]. Swartz et al. [12] used the TU approach for assessing the toxicity of PAH mixtures in sediments. The pres-

ent discussion is limited to the water phase only, but it applies to other phases, such as sediment and tissue of an organism. Equations 8 and 9 can be used after modification with parameters relevant to sediment or tissue. In water, a TU for a specific organism is defined as

$$TU_{W,i} = C_{W,i}/C^*_{W,i} \quad (8)$$

where $C_{W,i}$ is the measured concentration of chemical i in the water (mmol/L) and $C^*_{W,i}$ is the critical effect concentration for chemical i computed from Equation 5. The individual TUs (Eqn. 8) are then summed to compute the toxicity of the mixture:

$$TU = \sum_i TU_{W,i} \quad (9)$$

If the total TUs of the mixture are greater than or equal to 1, the mixture is predicted to be toxic. Using the LC50 as an example of an acute effect concentration, Equation 8 becomes

$$TU_{W,i} = C_{W,i}/LC50_i \quad (10)$$

Data compilation

More than 140 references were reviewed for water-column effect data resulting from exposure to MAHs or PAHs, both as single compounds and as mixtures. For data to be used in this analysis, the following four criteria needed to be met. First, a CTLBB must be available for the test organism. Second, the concentration of the test chemical(s) must be below water solubility. If a chemical is tested at a concentration above its water solubility, then the chemical is present as a pure phase, which may exert a different toxic mode of action. For single-chemical exposures, the appropriate liquid or solid solubility of the chemical is used, and for mixtures of chemicals that are solids as single compounds and liquids as mixtures, such as those in petroleum, the subcooled liquid solubility—that is, the solubility of the component if it were a liquid at the temperature of interest—is used. Third, the exposure concentrations must be constant with time, particularly for long-term exposures in which chronic effects are being tested. Diminishing or varying concentrations over time are difficult to interpret. One cannot assign a specific concentration to the observed effect. Fourth, for exposures to mixtures of chemicals, such as water-soluble fractions (WSFs) prepared from petroleum products, adequate characterization of the chemicals in the mixture is necessary. Petrogenic-source PAHs are known to have a large fraction of alkylated PAHs. Alkylated homologues are the parent PAHs substituted with carbon groups (e.g., methyl, ethyl, and propyl). Data from four nonweathered and weathered crude oils show that approximately 80 to 91% of the total PAH mass was present as alkylated material [13]. Similarly, data from nonweathered and weathered North Slope (AK, USA) crude oil showed that the alkylated PAHs accounted for more than 89 and 95%, respectively, of the total PAH mass [14]. In recent literature evaluating the toxicity of weathered oil, the heavy PAHs (e.g., fluoranthene and chrysene and their alkylated species) were present in exposure waters prepared from weathered oil, and their concentrations were shown to increase with weathering [4,5]. In the derivation of the sediment benchmarks for PAH mixtures, the importance of including the alkylated PAHs in a toxicity assessment was demonstrated [3]. In some cases, only using the 13 parent PAHs considered priority pollutants by the U.S. EPA to compute toxicity resulted in a 10-fold difference in the toxicity. Therefore, for petroleum exposures, in addition to measuring the water-soluble parent MAHs and PAHs, measurement for some representation of the alkylated homologues of parent compounds is required. Even the heavier PAHs that are of low water solubility will contribute to the toxicity of the mixture and should be included in the TU computation [15]. Omitting these heavy PAHs from the toxicity assessment could result in an underestimation of the toxicity.

For the analysis presented below, a pragmatic requirement for PAH measurements in water-column exposures was necessary to eliminate data sets that have too few measurements such that the toxicity can be severely underestimated. For a nonweathered petroleum source, representative compounds to be measured include BTEX, naphthalene, phenanthrene, and their alkylated homologues. For a weathered petroleum source, in addition to the light-molecular-weight PAH material, some contribution from the heavier PAHs and their alkylated homologues should be measured.

Physicochemical properties of hydrocarbons

A listing of chemicals used in the present paper is provided in Appendix 2. Although the focus is on MAHs and PAHs, for some data sets in which WSFs were prepared from petroleum sources, measurements for other hydrocarbons (e.g., alkanes) were provided. Because all hydrocarbons will exert a toxic potential [15], it is appropriate to include them in the computation of toxicity for the mixture. The chemical properties include molecular weight, $\log(K_{OW})$, solid solubility, and subcooled liquid solubility. The computer program SPARC [8] was used to compute the molecular weight and $\log(K_{OW})$. Recommended solid solubility and subcooled solubility values were taken from Mackay et al. [16–19]. Relationships between log(solubility) and $\log(K_{OW})$ were used to compute solid solubility and subcooled liquid solubility for chemicals that were not listed by Mackay et al.

RESULTS

Acute exposure effects (lethality)

Single compounds. For MAHs, a total of 164 data points (i.e., LC50/median effective concentration [EC50]) from 28 different species were used. The MAH toxicity values were taken from the data compilations used to develop the TLM [1,7]. For PAHs, a total of 140 data points from 20 different species were used. Data sets that comprise the acute lethality PAH toxicity database are summarized in Table 1. The observed and predicted LC50s are compared in Figure 1. The solid line represents the 1:1 relationship (i.e., where the observed and predicted LC50s are equal). The dashed lines represent the 90% confidence interval. The CL was computed as the 5th and 95th percentiles of the residuals (observed-predicted-effect concentrations). The model tends to slightly underestimate the toxicity. Based on this data analysis, with a 90% confidence level, the TLM predicts acute toxicity to within a factor of approximately three to five of the correct LC50, which is comparable to other models [20].

Mixtures. Three data sets that tested the toxicity of a mixture met the four selection criteria presented above. Two data sets were tests of the toxicity of WSFs prepared from crude and refined petroleum. The third used a prepared PAH mixture. All MAHs and PAHs measured in the mixture were included in the analysis. Details for each data set are provided in Table 2.

The toxicity of WSFs prepared from nonweathered and naturally weathered North Slope crude oil was measured [21].

Table 1. Data sets that comprise the acute lethality polycyclic aromatic hydrocarbon toxicity database

Species	No. of data points[a]	Concentration[b]	Test type[c]	Reference[d]
Artemia salina	6	U	S	[46]
	1	M	S	[47]
Chlamydomonas angulosa	4	U	S	[48]
Chlorella vulgaris	3	U	S	[48]
Cyprinodon variegatus	1	Unknown	R	[49]
	3	M	S	[50]
	2	U	S	[51]
	3	M	FT	[52,53]
Daphnia magna	15 (1)	U	S	[46,54–59]; U.S. EPA, unpublished data
	1	M	S, R	U.S. EPA, unpublished data
	8	M	S	[47,60–63]
	2	M	FT	[61,64]
Daphnia pulex	7	U	S	[65,66]
	1	M	S	[67]
Icialurus punctatus	1	M	FT	[68]
	1	M	S	[69]
Lepomis macrochirus	3	U	S	[57,70]
	2	M	FT	[44,64]
Leptochirus plumulosus	3	M	FT	U.S. EPA, unpublished data; U.S. EPA, unpublished data
	1	M	R	[71]
Menidia beryllina	(1)	U	R	U.S. EPA, unpublished data
	(1)	M	R	[44]
	1	U	S	[72]
Mysidopsis bahia	3	U	S	[52]; U.S. EPA, unpublished data
	1	U	R	U.S. EPA, unpublished data
	1	M	S	[61]
	8	M	FT	[44,53,61,72]; U.S. EPA, unpublished data
Neanthes arenaceodentata	3 (1)	U	S	[72,73]
Oithona davisae	6 (3)	M	S	[23]
Oncorhynchus mykiss	7	U	S	[57,74]
	1	M	S	[75]
	5	M	FT	[44,64,68,76]; G. DeGraeve, unpublished data, University of Wyoming, Laramie, Wyoming
	1	Unknown	R	[77]
Palaemonetes pugio	1	U	S	[72]; U.S. EPA, unpublished data
	2	U	R	[53]; U.S. EPA, unpublished data
	1	M	S	[78]
	1	M	R	[44]
	1	M	FT	[53]
	3	M	Unknown	[50]
Pimephales promelas	1	U	S	[79]
	5	M	S	[60,61,69,75,80]
	1	U	R	[81]
	8	M	FT	[68,76,82–84]; G. DeGraeve, unpublished data, University of Wyoming, Laramie, Wyoming
Tanytarsus dissimilis	2	U	S	[85]
Tetrahymena elliotti	1	M	S	[86]
Xenopus laevis	1	M	FT	[87]
	(1)	Unknown	R	[88]
Rhepoxynius abronius	7	Unknown	Unknown	[89]

[a] Numbers in parentheses represent data points limited by solubility (i.e., exposure concentration > aqueous solubility).
[b] M = measured; U = unmeasured or nominal.
[c] FT = flow-through; R = renewal; S = static.
[d] EPA = Environmental Protection Agency.

The total TU for the 100% WSF from nonweathered and weathered oils were 0.62 and 0.28, respectively. For the nonweathered oil, BTEX contribute significantly to the TU (~60% of the computed TU). This is not the case for weathered oil, in which BTEX account for less than 10% of the TUs. This analysis suggests that BTEX are important contributors to the toxicity of nonweathered oil compared to their toxic contribution in weathered oil, as was demonstrated by Neff et al. [13]. The effect of weathering on the toxicity of petroleum has been evaluated using the TLM [15].

The observed mortality versus total TU is presented in Figure 2A. Solid lines at a TU of 1.0 and 50% mortality are shown for guidance. The dashed lines represent the 5 and 95% uncertainties in the TLM predictions for *Pimephales promelas* and are computed using Equation 7 without the terms for ACR. For this data set, the dose–response pattern was correctly predicted by the TLM. The one data point where greater than 50% mortality occurs falls slightly to the left of the lower uncertainty limit of the TLM. All the other data points that have low observed mortality and corresponding low total TU fall to the left of the lower uncertainty bound, where low mortality is expected. The data set where greater than 50% mortality was observed was for nonweathered oil (Fig. 2A, open circles). A WSF prepared from nonweathered oil could

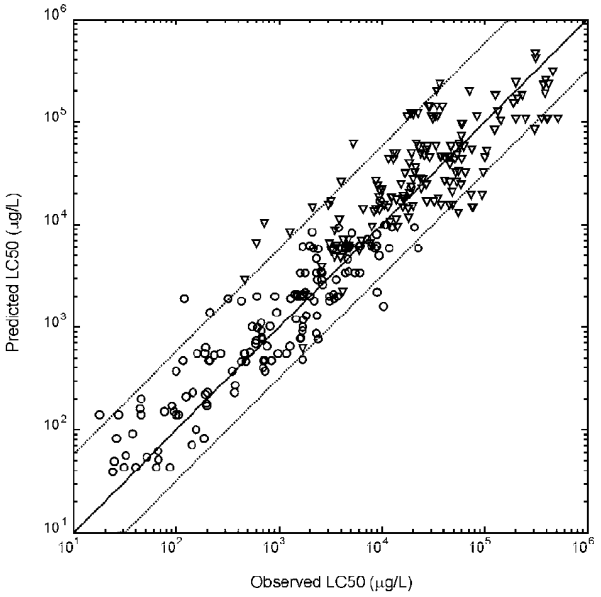

Fig. 1. Acute exposures to single compounds: Target lipid model predicted acute aqueous median lethal concentration (LC50) versus observed LC50 for monocyclic aromatic hydrocarbons (▽) and polycyclic aromatic hydrocarbons (○). Solid line represents a 1:1 relationship. Dashed lines represent the 90% confidence interval.

contain other, unmeasured water-soluble components that might contribute to the toxicity, which would result in a low computed TU.

States et al. [22] investigated the acute toxicity of WSFs prepared from no. 2 fuel oil and a solvent refined coal liquid to *Daphnia magna*. The total computed TU in the 100% WSF for no. 2 fuel oil was 0.36, suggesting that no toxic effects are expected. This result was in agreement with the reported no observed effects for the 100% WSF [22]. The total TUs computed for the 100% WSF from the coal liquid were 8.4, indicating that the 100% WSF would be predicted to be toxic. Reported data indicated that 0.25% WSF from the coal liquid was toxic. The equivalent TU at this dilution is 0.021, and at this level, no toxicity would be predicted from the aromatic hydrocarbons. In this case, the TLM predictions were not in agreement with the observed effects (low TU, high effect levels). States et al. [22], however, attributed the toxicity of the coal liquid to phenolic compounds, which are not type I narcotic chemicals and are not included in the TLM analysis. The phenolic compounds were present at significantly higher levels

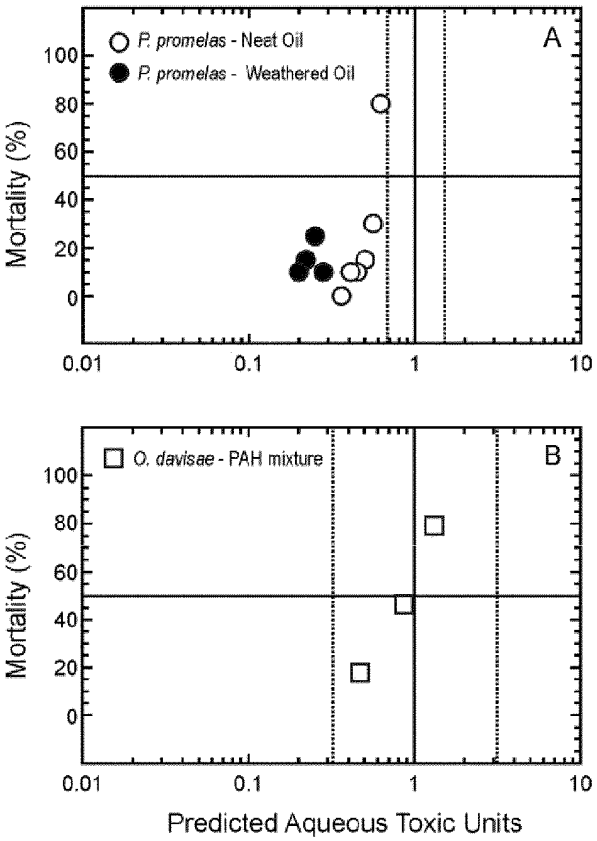

Fig. 2. Acute exposures to mixtures: Percentage mortality versus predicted aqueous toxic units. (**A**) Data for *Pimephales promelas* exposed to water-soluble fractions prepared from neat and weathered Alaska (USA) North Slope crude oil [21]. (**B**) Data for *Oithona davisae* exposure to water-soluble fraction prepared from a mixture of nine polycyclic aromatic hydrocarbons (PAHs) [23]. Solid lines represent 50% mortality (horizontal) and 1 toxic unit (vertical). Dashed vertical lines represent 5 and 95% uncertainty for each species.

on the coal liquid WSF (1,360 mg/L) compared to the no. 2 fuel oil WSF (1.7 mg/L). If the phenolic compounds are the main contributors to the toxicity, it is not surprising that the TLM did not predict the effects correctly, because they are not included in the TLM and TU calculation.

Barata et al. [23] tested the acute toxicity to the adult copepod *Oithona davisae* after 48 h of exposure to a mixture of nine PAHs. The observed mortality as a function of total TU

Table 2. Toxicity data sets for polycyclic aromatic hydrocarbon (PAH) mixtures[a]

PAH source	Species	PAH measured	Endpoint	Reference
Alaska (USA) North Slope crude oil (nonweathered and weathered)	*Pimephales promelas*	BTEX, biphenyl, 19 parent PAHs, 21 alkylated PAHs	48-h mortality	[21]
No. 2 fuel oil and coal liquid	*Daphnia magna*	Indan, tetralin, benzenes, naphthalenes	48-h immobilization	[22]
Prepared PAH mixture	*Oithona davisae*	Naphthalenes, phenanthrenes, pyrene, fluorene, fluoranthrene, dibenzothiophene	48-h mortality	[23]
Three prepared PAH mixtures	*Oryzias latipes*	Mixture 1: 3 parent PAHs; mixture 2: 3 dimethylated PAHs; mixture 3: oils sands extract (16 parent PAHs and their alkylated species)	After 18 d of exposure prevalence of BSD, % hatch, time to hatch, % normal larvae	[28]

[a] BSD = blue sac disease; BTEX = benzene, toluene, ethylbenzene, and xylene.

Table 3. Acute and chronic values used in the development of acute to chronic ratios (ACRs)[a]

Species and chemical	Chemical code[b]	Acute LC50/EC50 (μg/L)	Chronic endpoint	Chronic (μg/L)	ACR	Reference[c]
Cyprinodon variegatus						
Acenaphthene	PAH	3,100	Survival	710	4.36	[52]
Daphnia magna						
Fluoranthene	PAH	117	Growth	24.5	4.78	[44]
Phenanthrene	PAH	117	Survival and reproduction	96.4	1.21	[64]
Biphenyl	MAH	362		322	1.12	[90]
Methyl-*tert*-butyl ether	ALI	472,000	Not specified	42,000	11.2	[91]
Mysidopsis bahia						
Acenaphthene	PAH	466	Reproduction	286	1.63	[72]
Acenaphthene	PAH	460	Reproduction	64.0	7.19	[88]
Fluoranthene	PAH	30.5	Survival and reproduction	14.4	2.11	U.S. EPA, unpublished data
Fluoranthene	PAH	40.0	Survival and reproduction	15.9	2.52	U.S. EPA, unpublished data
Phenanthrene	PAH	27.1	Survival	8.13	3.33	U.S. EPA, unpublished data
Pyrene	PAH	28.3	Reproduction	4.53	6.24	U.S. EPA, unpublished data
Methyl-*tert*-butyl ether	ALI	187,000	Not specified	36,000	5.2	[91]
Oncorhynchus mykiss						
Phenanthrene	PAH	50.0	Survival and growth	6.32	7.91	[64]
Paratanytarsus sp.						
Acenaphthene	PAH	2,040	Survival, growth, and reproduction	411	4.96	[92]
Acenaphthene	PAH	2,040	Survival and growth	227	8.97	[92]; U.S. EPA, unpublished data
Pimephales promelas						
Acenaphthene	PAH	608	Growth	405	1.50	[82]; U.S. EPA, unpublished data
Acenaphthene	PAH	608	Growth	419	1.45	[82]
Naphthalene	PAH	7,900	Survival and growth	620	12.7	[76]
Fluoranthene	PAH	69.0	Survival and growth	15.0	4.60	[44]
Methyl-*tert*-butyl ether	ALI	980,000	Not specified	289,400	3.39	[91]
Brachionus calyciflorus						
Xylene	MAH	248,500		40,300	6.17	[93]
Ceriodaphnia dubia						
Toluene	MAH	3,750		2,780	1.35	[94]
Ethylbenzene	MAH	3,200		1,680	1.90	[94]
Selenastrum capricornutum						
Benzene	MAH	100,000	Biomass growth	8,300	12	[95]
Cyclohexane	ALI	9,317	Biomass growth	952	9.8	[96]
Methyl-*tert*-butyl ether	ALI	491,000	Biomass growth	103,000	4.8	[97]
Pentane	ALI	10,700	Biomass growth	2,040	5.2	[98]
Skeletonema costatum						
Ethylbenzene	MAH	7,700		4,500	1.7	[99]
Oncorhynchus gorbuscha						
Naphthalene	PAH	1,200	Growth	380	3.2	[100]

[a] Chronic endpoints are those effecting growth, reproduction, or mortality of an organism. Median lethal concentration (LC50) and median effective concentration (EC50) are the median concentrations producing 50% mortality and effects, respectively, in the test organisms.
[b] ALI = aliphatic hydrocarbon; MAH = monocyclic aromatic hydrocarbon; PAH = polycyclic aromatic hydrocarbons.
[c] EPA = Environmental Protection Agency.

is shown in Figure 2B. The dashed lines are the 5 and 95% uncertainties for *O. davisae* (Eqn. 7). For *O. davisae*, the dose response was correctly predicted by the TLM, where 50% mortality occurs at approximately 1.0 TU.

Chronic exposure effects

Single compounds. The analysis presented previously [1,2,7] suggests that the TLM, which was developed for chemicals that have a narcotic mode of toxic action, can be used to predict the acute toxicity of BTEX (and other MAHs) and PAHs. To convert the acute TLM endpoint to a chronic endpoint, an ACR is applied (Eqn. 6). A mean ACR of 5.09 was computed from the original database [1] that included BTEX and PAHs as well as other chemicals, such as chlorinated alkanes and MAHs (i.e., 1,2-dichloroethane and 1,2,4-trichlorobenzene). More than half the acute and chronic paired data sets were chlorinated compounds. Because petroleum products do not contained halogenated components, an analysis of the ACRs from nonhalogenated compounds is more appropriate.

A total of 29 paired data sets for aliphatic hydrocarbons, MAHs, and PAHs are available (Table 3), of which 17 are PAHs, six are MAHs, and six are aliphatic hydrocarbons. The distributions for each chemical class are shown in Figure 3A through C. The distributions are similar, spanning an ACR range of approximately 1 to 11. Because the distributions were similar, the data sets are combined (Fig. 3D). The geometric mean ACR from the combined data sets is 3.83.

The use of an ACR to convert an acute endpoint to a chronic endpoint does not imply that the toxic mode of actions for acute toxicity and chronic toxicity are the same. Rather, an ACR is a means of estimating the chronic toxicity from the acute toxicity of a chemical. It is used together with its variance to estimate the 5 and 95% CL.

Mixtures. Anderson et al. [24] investigated the hatching success of three marine species: *Cyprinodon variegatus*

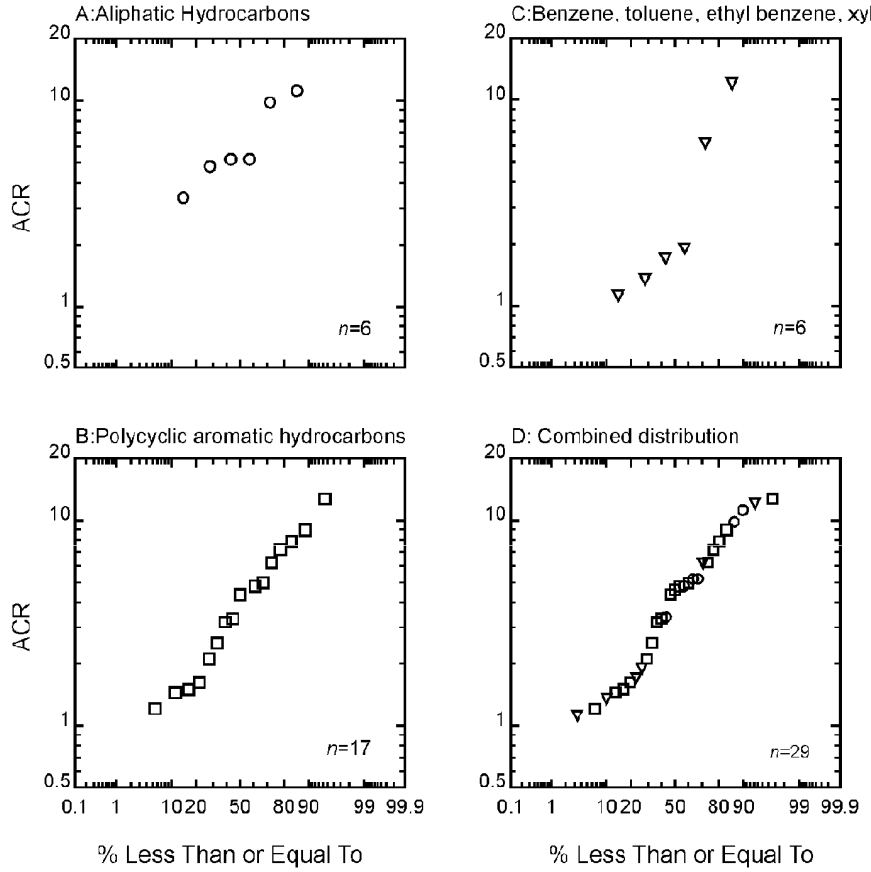

Fig. 3. Chronic effects of single compounds: Distribution of acute to chronic ratios (ACRs) for aliphatic hydrocarbons (**A**); polycyclic aromatic hydrocarbons (PAHs; **B**); benzene, toluene, ethylbenzene, and xylene (BTEX; **C**); and the combined data set (**D**).

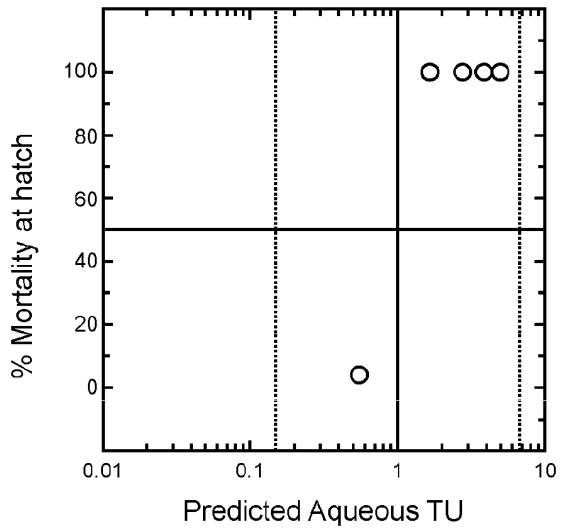

Fig. 4. Chronic effects of mixtures: Observed mortality versus predicted aqueous toxic units. Data are for *Cyprinodon variegatus* embryos exposed to water-soluble fraction prepared from no. 2 fuel oil [24]. Dashed vertical lines represent 5 and 95% confidence limits based on variations in critical target lipid body burden and acute to chronic ratio. TU = toxic unit.

(sheepshead minnow), *Fundulus heteroclitus* (mummichog), and *Fundulus similus* (longnose killifish) from chronic exposure to no. 2 fuel oil. A CTLBB is only available for *C. variegatus*. Concentrations of 31 hydrocarbons (11 alkanes, 8 MAHs, and 12 PAHs) in the WSF were reported [25]. With the exception of the lowest dilution, 100% mortality was observed in all exposures. The observed mortality versus predicted aqueous TUs is shown in Figure 4. For this data set, the TLM correctly predicted the observed effects: 100% mortality occurred at greater than 1 TU and low mortality occurred at less than 1 TU.

Moles [26] compared the sensitivity of 10 aquatic species to long-term exposure to WSFs prepared from Cook Inlet (AK, USA) crude oil. Organisms were exposed to various dilutions of the WSFs for 4 and 28 d. Although the concentrations of individual components in the WSFs were not measured, the 4- and 28-d LC50s were reported and used to compute ACRs. The computed ACRs ranged from 1 to 2.5 (data for which 4-d LC50 could not be computed were omitted from the analysis). These ACRs for crude oil are similar to the ACRs computed for individual chemicals that comprise petroleum and further support using a mean ACR of 3.8 for petroleum-related components.

Sublethal effects

Single-compound exposure. Recent literature suggests that exposure to PAHs during a fish's early life stage can result in a variety of sublethal effects (e.g., yolk sac edema, pericardial

edema, hemorrhaging, craniofacial and spinal deformities, lesions, defects in cardiac function, and reduced growth) [4–6,27,28]. Many of these symptoms are similar to those of blue sac disease (BSD), which is related to exposure to planar, halogenated aromatic compounds, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [29]. These sublethal effects were not included in the development of the ACRs. Therefore, it is necessary to determine if the TLM is protective of these effects.

Fourteen data sets were identified for analysis; however, only six data sets satisfied all four criteria discussed above. In all 14 data sets, the chemical exposure concentrations were less than the chemical's water solubility, and species-specific CTLBBs were available for all test organisms. For some of the exposures, the reported test concentrations were nominal rather than measured values, and the exposure conditions were static (i.e., not constant) rather than flow-through or static renewals (i.e., constant). Following the spirit of U.S. EPA water-quality criteria guidelines [9], the data sets were given a ranking number, where 1 represents data of the lowest quality (nominal concentrations and static conditions) and 4 represents data of the highest quality (measured concentrations and flow-through conditions). Data with a ranking number of 2 or 3 were based on measured concentrations determined under static/static-renewal test conditions. Data with a ranking number of 3 or 4 are more comparable with the TLM, because the effect concentrations were measured concentrations and relatively stable throughout the test. Six data sets were given a ranking number of at least 3.

Data were available for five fish species and nine chemicals (Table 4). The reported concentrations included lowest-observed-effect concentrations (LOECs), no-observed-effect concentrations (NOECs), and observed-effect concentrations (OECs). The TLM chronic endpoint, C^*_W, and corresponding $C^*_{W,5\%}$ and $C^*_{W,95\%}$values are provided. The observed effect concentrations and TLM chronic endpoints are compared in Figures 5 through 7. The symbols represent the TLM endpoint computed using species-specific CTLBB and the average ACR. The upper and lower bars around the symbol represent the $C^*_{W,95\%}$ and $C^*_{W,5\%}$, respectively, and are computed based on the uncertainty in the CTLBB and the ACR (Eqn.7). Because the same ACR data set is used to compute the $C^*_{W,95\%}$ and $C^*_{W,5\%}$ values for all organisms, the difference in the degree of the uncertainty is dependent on the uncertainty associated with the CTLBB. A large uncertainty results from having a small acute toxicity data set from which the CTLBB is determined. More acute toxicity would be needed to confirm the CTLBB and lower the uncertainty.

The results for early life-stage Japanese medaka (*Oryzias latipes*) exposed to different aromatic hydrocarbons are shown in Figure 5. The lowest-effect concentration was a 17-d EC50 of 10 μg/L of retene for BSD symptoms [30]. The highest-effect concentration was a LOEC of 200 μg/L of dibenzothiophene for endpoints of percentage hatch, time to hatch, prevalence of BSD, and percentage normal larvae [28]. In comparison, the TLM chronic endpoints range from 4.6 μg/L for retene and 240 μg/L for dibenzothiophene, which are close to the observations. For benzo[*a*]anthracene, the tested concentrations were greater than its water solubility of 11 μg/L; therefore, the data were not considered [28]. No effects were observed for phenanthrene and 3,6-dimethylphenanthrene at any of the concentrations tested (e.g., up to 200 μg/L) [28]. For these two PAHs, the TLM endpoints were within the tested concentration ranges, and the TLM would have predicted effects to occur. Thus, the TLM predictions are protective for these two PAHs.

Several studies investigated the impacts of exposing early life-stage rainbow trout (*Oncorhynchus mykiss*) to PAHs (Fig. 6). The 27-d LC50s based on mortality and teratogenesis for naphthalene and phenanthrene were 110 and 40 μg/L, respectively [31]. Another study with phenanthrene reported significant mortality and abnormal larvae after 12 d of exposure and the prevalence of BSD after 22 d of exposure at a concentration of 500 μg/L [32]. The TLM chronic endpoint for phenanthrene is 70 μg/L and is consistent with observations. The TLM prediction of 800 μg/L for naphthalene is well above the observation of 110 μg/L. The observed effect concentration, however, is only slightly less than the $C^*_{W,5\%}$ of 170 μg/L.

Brinkworth et al. [27] exposed early developmental stages of rainbow trout to many different regimes of retene. One condition was a constant exposure to 9 μg/L of retene. Additional exposures were either not continuous or at concentrations greater than the water solubility of retene (i.e., 16 μg/L). After 45 d of exposure to 9 μg/L of retene, 85% of the larvae showed signs of BSD. The TLM chronic endpoint for retene is 2.4 μg/L.

The LOEC for morphological abnormalities in trout alevins resulting from exposure to benzo[*a*]pyrene was 0.21 μg/L [33]. At this concentration, the number of effects observed in organisms was significantly different than in controls; however, no statistical differences were found at higher concentrations of 0.37 and 1.48 μg/L of benzo[*a*]pyrene. Statistical differences were reported at benzo[*a*]pyrene concentrations of 0.21, 2.4, and 2.99 μg/L. Even though the dose response for benzo[*a*]pyrene is inconsistent, the LOEC of 0.21 μg/L is retained, both because it is a very low concentration and because the U.S. EPA included it in their assessment of teratogenic effects from PAHs [3]. The TLM chronic endpoint for benzo[*a*]pyrene is 1.9 μg/L (CL, 0.36–11 μg/L), which is in the range of where effects were observed.

Middaugh et al. [34] investigated the teratological effects of naphthalene on the embryos of Inland silverside (*Menidia beryllina*). The LOEC was 550 μg/L for cardiovascular effects and 2,350 μg/L for craniofacial and skeletal effects. The TLM chronic endpoint for naphthalene is 3,900 μg/L, which is greater than the lowest reported LOEC (Fig. 7). For fathead minnow (*P. promelas*), heritable reductions in larval survival were reported at a LOEC of 1 μg/L [35]. The TLM chronic endpoint of 3.6 μg/L (CL, 0.83–16 μg/L) is greater than the LOEC, but the LOEC is within the CL (Fig. 7). For zebrafish (*Brachydanio rerio*), the reported LOEC for sublethal effects of deformities and hemorrhages from exposure to benzo[*k*]fluoranthene is 0.72 μg/L [36]. The corresponding NOEC for growth is 0.23 μg/L. The TLM chronic endpoint of 3.8 μg/L (CL, 0.47–30 μg/L) is greater than the reported effect values.

The TLM chronic endpoint was higher than the reported LOEC/OEC for eight of the 15 cases, suggesting that the TLM chronic endpoint may not be protective of these sublethal effects. When the uncertainty is considered, however, then with the exception of one naphthalene data point for rainbow trout, the $C^*_{W,5\%}$ values are below the reported LOEC for sublethal effects.

Mixtures. Several laboratory studies demonstrated that long-term exposure to petroleum results in various sublethal effects in early life-stage pink salmon (*Oncorhynchus gorbuscha*) and Pacific herring (*Clupea pallasi*) [4,5,37]. Data from these studies could not be analyzed, because the chemical exposure concentrations were not constant and drastically decreased (by

Table 4. Comparison of target lipid model (TLM) chronic endpoints to no-observed-effect concentrations (NOECs), lowest-observed-effect concentrations (LOECs), and observed-effect concentrations (OECs; sublethal effects) from early life-stage tests for single polycyclic aromatic hydrocarbon exposures

Chemical	Effects[a]	Observed concentration	TLM chronic endpoint	$C^*_{w,5\%}$[b]	$C^*_{w,95\%}$[c]	Ranking[d]	Reference
Oryzias latipes (Japanese medaka)							
Toluene	Deformation of eyes, embryonic malformation, heart abnormality	LOEC = 41 mg/L; NOEC = 16 mg/L	12 mg/L	0.28 mg/L	560 mg/L	2	[101]
Dibenzothiophene	18 d of exposure: Hatching success; time to hatch; BSD symptoms; % normal	LOEC = 200 μg/L; NOEC = 100 μg/L	240 μg/L	5 μg/L	11,000 μg/L	1	[28]
4,6-Dimethyldibenzothiophene	18 d of exposure: % normal larvae	LOEC = 12.5 μg/L	32 μg/L	0.66 μg/L	1,500 μg/L	1	[28]
Denzo[*a*]anthracene	Concentrations of benzo[*a*]anthracene tested were above it water solubility; data not valid						
Phenanthrene	No effects observed at highest concentration tested (200 μg/L)		135 μg/L	2.9 μg/L	6,330 μg/L	1	[28]
3,6-Dimethylphenanthrene	No effects observed at highest concentration tested (200 μg/L)		24 μg/L	0.5 μg/L	1,200 μg/L	1	[28]
Retene	17-d EC50 for BSD	10 μg/L	4.6 μg/L	0.10 μg/L	210 μg/L	4	[30]
7,12-Dimethylbenz[*a*]anthracene	18 d of exposure: Time to hatch	LOEC = 12.5 μg/L	2.8 μg/L	0.06 μg/L	140 μg/L	1	[28]
Oncorhynchus mykiss (rainbow trout)							
Naphthalene	27-d LC50 based on mortality and teratogenesis	110 μg/L	880 μg/L	170 μg/L	4,500 μg/L	4	[31]
Phenanthrene	27-d LC50 based on mortality and teratogenesis	40 μg/L	70 μg/L	10.4 μg/L	470 μg/L	4	[31]
Phenanthrene	Significant mortality and abnormal larvae at 12 d (hatch); BSD symptoms after 22 d (swim up)	500 μg/L	70 μg/L	10.4 μg/L	470 μg/L	1	[32]
Retene	49 d of exposure: 85% BSD; 59% hemorrhaging; 25% yolk sac edema; <5% mortality	9 μg/L	2.4 μg/L	0.44 μg/L	13 μg/L	4	[27]
Benzo[*a*]pyrene	36 d of exposure: Alevin abnormalities	LOEC = 0.21–2.4 μg/L	1.9 μg/L	0.36 μg/L	11 μg/L	3	[33]
Menidia beryllina (inland silverside)							
Naphthalene	2- to 4-cell stage: Cardiovascular effects	LOEC = 550 μg/L	3,900 μg/L	240 μg/L	62,000 μg/L	2	[34]
	2- to 4-cell stage: Craniofacial and skeletal effects	LOEC = 2350 μg/L; NOEC = 550 μg/L					
Pimephales promelas (fathead minnow)							
Benzo[*a*]pyrene	Heritable reductions in larval survival	LOEC = 1 μg/L	3.6 μg/L	0.83 μg/L	16 μg/L	1	[35]
Brachydanio rerio (zebrafish)							
Benzo[*k*]fluoranthene	42 d of exposure: Deformities and hemorrhages	LOEC = 0.72 μg/L	3.8 μg/L	0.47 μg/L	30 μg/L	4	[36]

[a] BSD = blue sac disease; LC50 = median lethal concentration.
[b] $C^*_{w,5\%}$ = lower confidence limit for water concentration.
[c] $C^*_{w,95\%}$ = upper confidence limit for water concentration.
[d] The higher the ranking, the more criteria that the data set met.

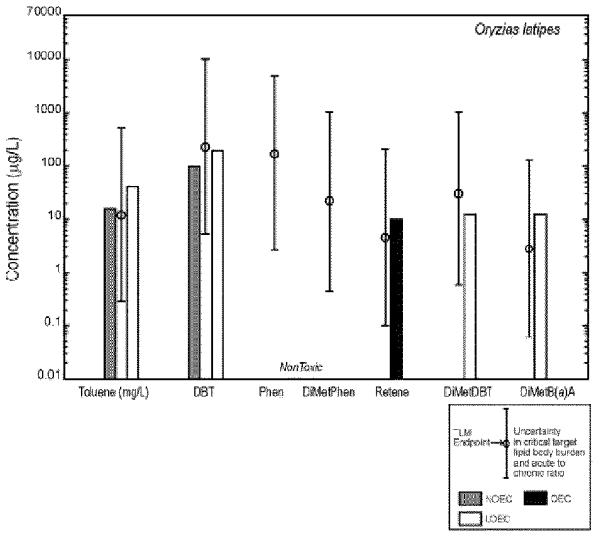

Fig. 5. Sublethal effects of single compounds: Comparison of observed-effect concentration (OEC)/lowest-observed-effect concentration (LOEC)/no-observed-effect concentration (NOEC) observed from early life-stage fish exposures (*Oryzias latipes*) to single compounds to target lipid model (TLM) chronic effect concentrations. The symbols represent the TLM chronic endpoint. The lines associated with the TLM chronic endpoints represent 5 and 95% confidence limits based on variations in critical target lipid burden body (CTLBB) and acute to chronic ratio (ACR). See Table 4 for references. Phen = phenanthrene; DBT = dibenzothiophene; DiMetPhen = 3,6-dimethylphenanthrene; DiMetDBT = 4,6-dimethyldibenzothiophene; DiMetB(a)A = 7,12-dimethylbenzo[*a*]anthracene).

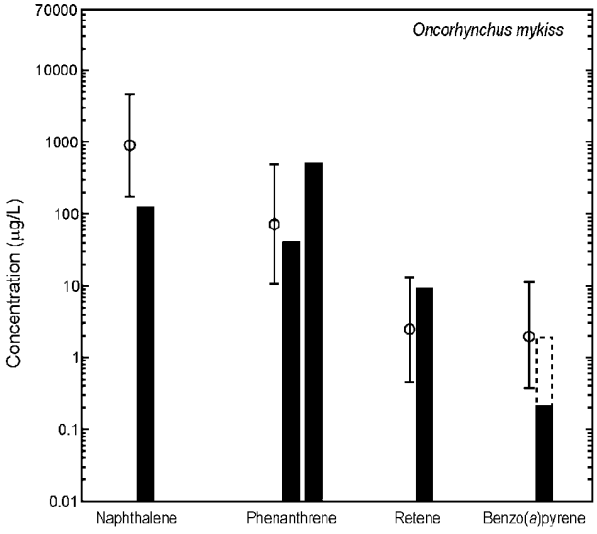

Fig. 6. Sublethal effects of single compounds: Comparison of observed-effect concentrations observed from early life-stage fish exposures (*Oncorhynchus mykiss*) to single compounds to target lipid model (TLM) chronic effect concentrations. The symbols represent the TLM chronic endpoint. The lines associated with the TLM chronic endpoints represent 5 and 95% confidence limits. See Table 4 for references and Figure 5 for symbol identification.

orders of magnitude) during the exposure period. Because of the variable exposure concentrations, linking the observed effects to the exposure concentrations was not possible.

Rhodes et al. [28] determined the effects of three PAH mixtures on embryonic development of early life-stage medaka (Table 2). The exposure system was static renewal, and nominal concentrations were reported for the two mixtures prepared from three PAHs. For the oil sands extract, the concentrations of PAHs were measured. Figure 8 presents a comparison of the incidence of effects as a function of TLM-predicted total TUs for the three different mixtures. Solid lines at 50% effects and a TU of one are shown for guidance. The filled symbols indicate the response was significantly different than the control response. For the parent PAH mixture, the predicted total TUs were greater than 1 (i.e., 1.2 and 2.4) in the two highest exposure concentrations, and effects would be predicted to occur at these concentrations. The most sensitive endpoints to the parent PAH mixture were percentage abnormal larvae and percentage egg hatch, which were significantly different at the highest exposure concentration that had an equivalent total TU of 2.4. For the dimethylated PAH mixture, the predicted total TUs were greater than one in four exposures and ranged from 1.5 to 12. The most sensitive endpoint to the dimethylated PAH mixture was percentage egg hatch, for which significant effects were observed in the three highest exposures, where the total TUs ranged from 3 to 12. The oil sands extract exposures had total TUs ranging from 0.2 to 3.9. The most sensitive endpoint to the extract was hatch length, for which all exposures were impacted. This is an interesting result, because no impact on hatch length was observed for other mixtures. The percentage abnormal larvae and BSD score were significantly different from controls only in the two highest exposure concentrations, where the total TUs were greater than 1. The dose response for percentage egg hatch was sporadic.

For the parent and dimethylated PAH mixtures, the TLM and TU approach gave reasonable predictions for the most sensitive endpoints. For the exposures that had significant effects as compared to controls, the total TUs were greater than two. Some exposures had total TUs of greater than 1 but with no effects observed. For the oil sands extract, the TLM and TU approach did not accurately predict the most sensitive endpoint of hatch length. The model did have a correct prediction for abnormal larvae and BSD score.

The 5 and 95% uncertainty bounds for the medaka TU prediction were omitted from Figure 8. The computed uncertainty bounds are very large, ranging from a TU of 0.02 to 50, and as such, they do not provide a good representation of the true uncertainty. The large uncertainty in predictions for medaka (i.e., wide range in $C^*_{W,5\%}$ and $C^*_{W,95\%}$) results from the large k_Z value of 4.47. The data set used to compute the CTLBB only consisted of five data points. With such a high k_Z value, it is almost certain that the $C^*_{W,5\%}$ will be lower than the observed effect concentration. Medaka is a commonly used test organism, and additional acute toxicity data are needed to better quantify the statistics of the CTLBB.

DISCUSSION

HC5 for decision making

The method for computing $C^*_{W,5\%}$ for a specific species based on the CTLBB and its associated uncertainty was presented (Eqn.7). This method also can be used to compute a hazard concentration for 95% species protection (i.e., HC5) by using the probability distribution of the CTLBB (Fig. 9) to obtain the geometric mean CTLBB (119 µmol/g octanol). Then, the computed endpoint is the aqueous concentration that protects 95% of species for which a CTLBB is available.

The resulting equation to compute the chronic HC5s for PAHs is

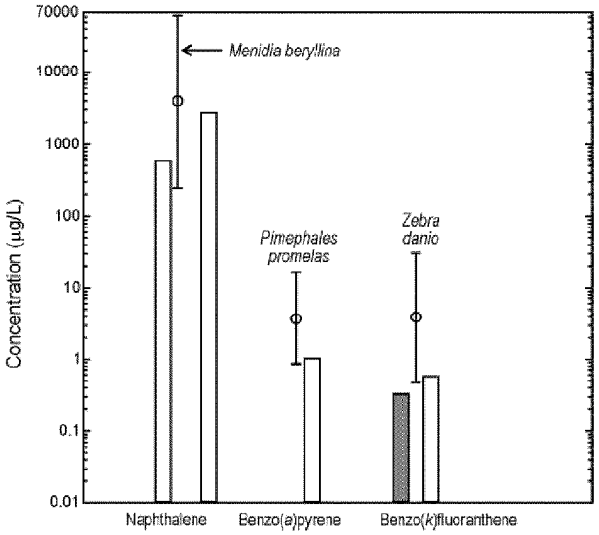

Fig. 7. Sublethal effects of single compounds: Comparison of observed effect concentrations observed from early life-stage fish exposures to single compounds to target lipid model (TLM) chronic effect concentrations. The symbols represent the TLM chronic endpoint. The lines associated with the TLM chronic endpoints represent 5 and 95% confidence limits. See Table 4 for references and Figure 5 for symbol identification.

$$\log(\text{HC5}) = (-0.936)\log(K_{OW}) + \log(52.9) - \log(3.83) - 2.3\sqrt{\{0.000225[\log(K_{OW})^2]\} + 0.105 + 0.112} \quad (11)$$

The resulting equation to compute the chronic HC5s for MAHs is

$$\log(\text{HC5}) = (-0.936)\log(K_{OW}) + \log(92.7) - \log(3.83) - 2.3\sqrt{\{0.000225[\log(K_{OW})^2]\} + 0.105 + 0.112} \quad (12)$$

where the appropriate Δc_i corrections (Appendix 1) are included.

The 95% confidence sample size–dependent extrapolation factor (k_Z = 2.3) is based on the number of ACRs (29) rather than the number of CTLBBs (47) to ensure that the results are conservative. A summary of predicted chronic HC5s for some common MAHs and PAHs is provided in Table 5.

A comparison of the aqueous HC5s and 27 NOECs based on growth, reproduction, and mortality endpoints for seven different PAHs is presented in Table 6 and Figure 10. In addition, the five NOECs for sublethal effects (Table 4) are shown in Figure 10. Two NOECs fall below the HC5 line. Both of these NOECs are for phenanthrene and range from 5 to 5.5 µg/L, which is considerably lower than NOECs for other compounds. The excursion of two data points below the HC5 is consistent with the goal of a 95% protection level (i.e., 93.8% (30/32)). The HC5 derived from the TLM is very close to the expected level of protection for growth, reproduction, mortality, and sublethal effects. Therefore, these values are appropriate for use as numeric, chemical-specific benchmarks, and they can be used to assess the ecological risk of contaminated sediments and/or establish safe levels for cleanup activities. Of course, one needs to consider the assumptions of the TLM and the conditions that can impact the toxicity of MAHs and PAHs (e.g., constant exposure) to determine the appropriateness of applying these guidelines to a specific location.

Table 5. Predicted chronic 5% hazard concentrations (HC5s) for monocyclic and polycyclic aromatic hydrocarbons

Chemical	$\log(K_{OW})$	HC5 µmol/L	HC5 µg/L
Benzene	1.943	30.892	2,413
Toluene	2.438	10.600	977
o-Xylene	2.946	3.534	375
Ethylbenzene	3.006	3.104	330
m-Xylene	3.032	2.935	312
p-Xylene	3.051	2.816	299
Naphthalene	3.256	1.033	132
Acenaphthylene	3.436	0.700	107
1-Methylnaphthalene	3.781	0.332	47.2
2-Methylnaphthalene	3.789	0.326	46.3
Acenaphthene	3.878	0.269	41.5
Fluorene	3.93	0.240	39.9
Biphenyl	3.936	0.415	64.0
1,3-Dimethylnaphthalene	4.257	0.118	18.5
2,6-Dimethylnaphthalene	4.27	0.115	18.0
Dibenzothiophene	4.34	0.0988	18.2
1-Methylfluorene	4.37	0.0926	16.7
Anthracene	4.546	0.0633	11.3
2,3,5-Trimethylnaphthalene	4.57	0.0601	10.2
Phenanthrene	4.584	0.0583	10.4
9-Methylanthracene	4.996	0.0239	4.59
1-Methylphenanthrene	5.036	0.0219	4.21
Pyrene	5.126	0.0180	3.64
Fluoranthene	5.19	0.0157	3.17
Benzo[*a*]anthracene	5.744	0.00471	1.08
Chrysene	5.782	0.00434	0.99
Retene	6.12	0.00208	0.488
Indeno[1,2,3-*cd*]pyrene	6.158	0.00192	0.530
Benzo[*b*]fluoranthene	6.341	0.00129	0.325
Benzo[*k*]fluoranthene	6.4	0.00113	0.286
Benzo[*a*]pyrene	6.409	0.00111	0.281
7,12-Dimethylbenzo[*a*]anthracene	6.42	0.00109	0.278
Perylene	6.447	0.00102	0.258

Range of application

The TLM was validated for use in predicting the toxicity of petroleum-related compounds. Once validated, the HC5s were derived for use in assessing the impacts of residual levels of these compounds on the aquatic community. It is impractical and probably impossible to develop guidelines that address every situation that may occur in the environment. The following are some conditions and assumptions of the TLM that can impact the toxicity of petroleum-related components that were not addressed in the present research but are important to consider when determining the effects from petroleum.

The TLM assumes that octanol is a good surrogate for organism lipid and that the K_{OW} describes the partitioning of the chemical between the water and the organism lipid. Some data suggest that a toxicity cutoff exists for compounds with $\log(K_{OW})$ greater than approximately 5.5 (i.e., compounds with $\log(K_{OW}) \geq 5.5$ have similar toxicity), and toxicity assessments have included a K_{OW} cutoff and rely on the membrane–water partition coefficient, K_{MW}, to be the descriptor for toxicity [38]. The K_{MW} has been shown to be a better surrogate than the K_{OW} for describing the partitioning of a chemical into the organism lipid membrane [39–41]. The $\log(K_{MW})$–$\log(K_{OW})$ relationship is linear until a $\log(K_{OW})$ of approximately 5.5, at which point

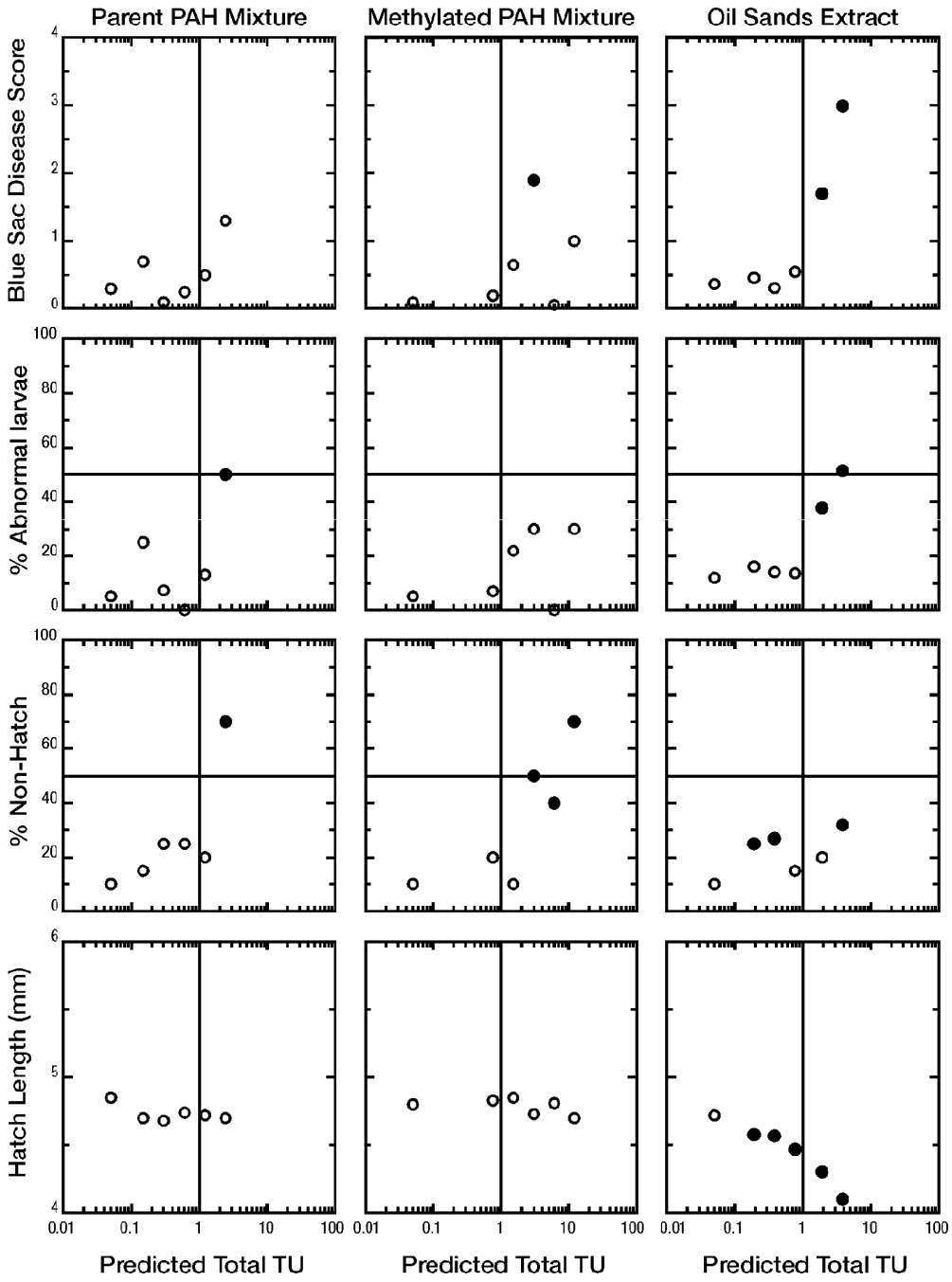

Fig. 8. Sublethal effects of mixtures: Comparison of percentage effects versus predicted total toxic units for three prepared mixtures exposed to early life-stage *Oryzias latipes* [28]. Solid lines represent 50% mortality (horizontal) and 1 toxic unit (TU; vertical). Filled symbols indicate the response was significantly different than the control response. PAHs = polycyclic aromatic hydrocarbons.

the $\log(K_{MW})$ no longer increases linearly with $\log(K_{OW})$ [41]. The TLM, however, ignores a cutoff and relies on the K_{OW} to be the descriptor for the toxicity, both because a cutoff point for PAHs has not been confirmed or identified and because toxicity from PAHs with $\log(K_{OW})$ greater than 5.5 has been observed. In a recent paper by Jonker and Van Der Heijden [42], PAHs with $\log(K_{OW})$ of up to 7.5 were shown to bioaccumulate in worms, and the relationship between log(bioconcentration factor) and $\log(K_{OW})$ was linear. The TLM would not have correctly predicted the toxicity if a cutoff or the K_{MW} had been used in the analysis. While some evidence indicates that a toxicity cutoff does exist for narcotic chemicals, a universal cutoff has yet to be identified, because it varies for each chemical. It is recommended that this methodology be used for chemicals that are similar to those used in this derivation. For practical purposes, an upper bound of $\log(K_{OW})$ would be 6.4, the highest $\log(K_{OW})$ for chronic experimental data that are in agreement with the predicted HC5s.

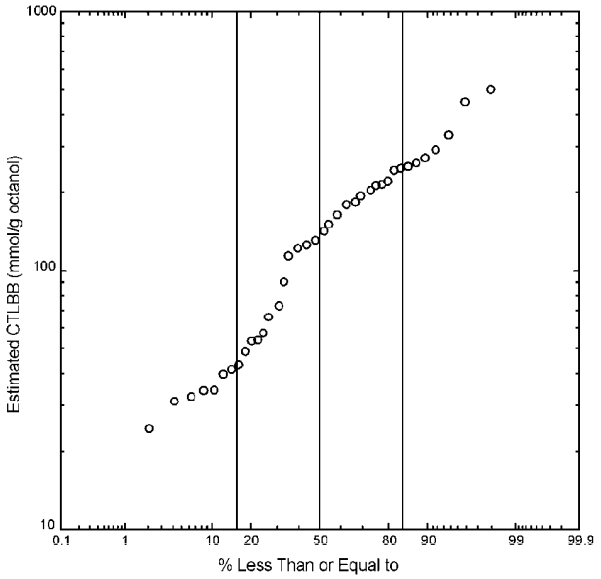

Fig. 9. Probability distribution of critical target lipid body burdens. CTLBB = critical target lipid body burden.

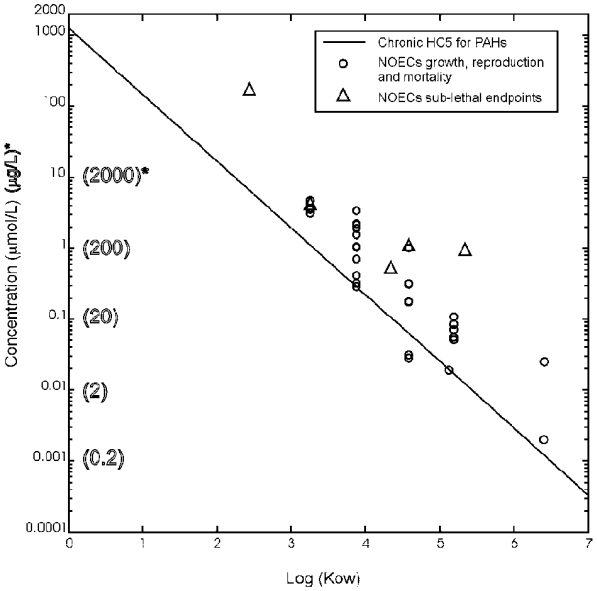

Fig. 10. Chronic HC5 (the hazard concentration affecting 5% of the test species) concentration for polycyclic aromatic hydrocarbons (PAHs) versus log octanol–water partition coefficient (K_{OW}). The HC5 is based on the fifth percentile critical target lipid body burdens for all species in the database. The units (µg/L) were computed using a molecular weight of 200 g/mol. The no-observed-effect concentrations (NOECs) for growth, reproduction, and mortality endpoints for PAHs are denoted as ○ (Table 6); the NOECs based on sublethal endpoints are denoted as △ (Table 4).

The MAHs and PAHs were assumed to be the primary causative agents of petroleum toxicity. There may, however, be other water-soluble components of petroleum that could exert toxicity. The TLM could be applied to any chemical, but the toxicity computed would be considered as the chemical's baseline or minimum toxicity. Additionally, some PAHs exert photoenhanced toxicity after accumulation in the tissues of organisms and exposure to ultraviolet radiation. Photoinduced

Table 6. Polycyclic aromatic hydrocarbon (PAH) no-observed-effect concentration (NOEC; growth, reproduction, mortality) toxicity data for various species[a]

Chemical	Species	Endpoint	Endpoint (µg/L)	Reference[b]
Naphthalene	*Daphnia magna*	21-d NOEC, reproduction	600	[102]
	Oncorhynchus kisutch	40-d NOEC, larval growth	370	[103]
	Pimephales promelas	30-d NOEC, larval growth	450	[76]
Acenaphthene	*Paratanytarsus* sp.	26 d NOEC, survival/growth/reproduction	295	[92]
	Paratanytarsus sp.	26-d NOEC, survival/growth/reproduction	164	[92]
	Pimephales promelas	32-d NOEC, growth	109	[81]
	Pimephales promelas	32-d NOEC, growth	50	[81]
	Pimephales promelas	32- to 35-d NOEC, growth	332	[82]
	Pimephales promelas	32- to 35-d NOEC, growth	345	[82]
	Pimephales promelas	32-d NOEC, survival	64	[104]
	Americamysis bahia	35-d NOEC, survival	240	[72]
	Americamysis bahia	25-d NOEC, survival/reproduction	44.6	U.S. EPA, unpublished data
	Cyprinodon variegatus	28-d NOEC, survival	520	[52]
Phenanthrene	*Daphnia magna*	21-d NOEC, reproduction	180	[105,106]
	Daphnia magna	21-d NOEC, survival	56	[105,106]
	Daphnia magna	21-d NOEC, growth	32	[105,106]
	Daphnia magna	21-d NOEC, reproduction	57	[64]
	Brachydanio rerio	42-d NOEC, growth	56	[105,106]
	Oncorhynchus mykiss	90-d NOEC, survival/growth	5	[64]
	Americamysis bahia	32-d NOEC, survival	5.5	U.S. EPA, unpublished data
Fluoranthene	*Brachydanio rerio*	41-d NOEC, growth	22	[107]
	Daphnia magna	21-d NOEC, growth	17	[44]
	Pimephales promelas	32-d NOEC, survival/growth	10.4	[44]
	Americamysis bahia	31-d NOEC, survival/reproduction	11.1	[44]
Pyrene	*Americamysis bahia*	28-d NOEC, reproduction	3.82	U.S. EPA, unpublished data
Benzo[*k*]fluoranthene	*Brachydanio rerio*	42-d NOEC, growth	0.23	[36]
Benzo[*a*]pyrene	*Brachydanio rerio*	42-d NOEC, growth	6.3	[106]

[a] Only measured data are considered.
[b] EPA = Environmental Protection Agency.

toxicity can be orders of magnitude greater than baseline toxicity (i.e., narcosis) [43–45].

The physicochemical properties of the MAHs and PAHs used in the present research were determined at 25°C. These parameters vary with temperature. For example, organic compounds usually are less soluble in colder temperatures. Therefore, the computed guidelines would be conservative for temperatures less than 25°C.

CONCLUSION

It has been demonstrated that the TLM can be used to assess the acute and chronic toxicity of petroleum-related components in the water column and that the use of a single ACR to relate the acute toxicity to an equivalent chronic toxicity is appropriate without consideration of the toxicity mechanism. Within the uncertainty of the model, the TLM has been shown to be protective of sublethal effects that result from exposure to low levels of PAHs during an organism's early life stages. The TLM and the additivity of TUs can be used to predict the toxicity of mixtures of chemicals present in petroleum. Toxic units are a means of normalizing the toxicity of different chemicals. It should be noted that the available toxicity data for mixtures that met the criteria for inclusion, both on an acute and a chronic basis, are limited. Additional research is needed to determine the toxicity of mixtures of chemicals resulting from exposure to petroleum regarding species for which a CTLBB is available. These data would further validate that the TLM and TU concept are appropriate for predicting the toxicity.

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APPENDIX I. Revised regression coefficients for the target lipid model

Species scientific same	Species common name	Environment	Habitat	*n*	k_Z[a]	$\log(C_L^{*b})$	SE^c $(\log(C_L^{*b}))$	C_L^{*b} (μmol/g octanol)	SE^c (C_L^{*b}) (μmol/g octanol)
Oncorhynchus gorbuscha	Pink salmon	Both	Water column	18	2.57	1.389	0.029	24.5	1.6
Rhepoxyinus abronius	Amphipod	Salt water	Infauna	7	3.59	1.494	0.158	31.2	12.5
Hyalella azteca	Amphipod	Freshwater	Epibenthic	3		1.512		32.5	
Mysidopsis bahia	Mysid	Salt water	Epibenthic	29	2.30	1.535	0.087	34.3	7.1
Chlamydomonas reinhardtii	Green algae	Many different	Variety	9	3.19	1.536	0.141	34.4	12.1
Chironomus tentans	Midge	Freshwater	Infauna	3	—	1.599		39.7	
Eohaustorius estuarius	Amphipod	Salt water	Infauna	2	—	1.617		41.4	
Lepiocheirus plumulosus	Amphipod	Salt water	Epibenthic, infauna	4	5.49	1.634	0.203	43.1	23.8
Selenastrum capricornutum	Green algae	Freshwater	Variety	24	2.41	1.687	0.091	48.6	10.5
Portunus pelagicus	Flower crab, sand crab	Salt water	Epibenthic	4	5.49	1.727	0.203	53.3	29.4
Ampelisca abdita	Amphipod	Salt water	Infauna	1	—	1.731		53.8	
Palaemonetes pugio	Grass shrimp	Salt water	Epibenthic, water column	10	3.06	1.758	0.127	57.3	17.9
Oncorhynchus mykiss	Rainbow trout	Freshwater	Infauna	47	2.13	1.820	0.067	66.1	10.4
Jordanella floridae	American flag-fish	Salt water	Water column	18	2.57	1.827	0.101	67.1	16.3
Ictalurus punctatus	Channel catfish	Freshwater	Epibenthic	7	3.59	1.864	0.149	73.1	27.4
Daphnia pulex	Cladoceran	Freshwater	Water column	26	2.36	1.957	0.089	90.6	19.2
Cyprinodon variegatus	Sheepshead min-now	Salt water	Epibenthic, water column	24	2.41	2.055	0.094	114	25.5
Daphnia magna	Cladoceran	Freshwater	Water column	117	1.93	2.062	0.050	115	13.4
Pimephales promelas	Fathead minnow	Freshwater	Water column	185	1.87	2.087	0.044	122	12.5
Danio rerio	Zebrafish	Freshwater	Water column	18	2.57	2.099	0.101	126	30.5
Rana catesbian	American bull-frog	Freshwater	Water column	5	4.47	2.101	0.174	126	57.1
Oryzias latipes	Japanese medaka	Freshwater	Water column	5	4.47	2.104	0.174	127	57.5
Lepomis macrochirus	Bluegill	Freshwater	Water column	70	2.04	2.117	0.059	131	18.1
Orconectes immunis	Crayfish	Freshwater	Epibenthic	6	3.93	2.137	0.160	137	56.0
Oithona davisae	Copepod	Salt water	Epibenthic	9	3.19	2.151	0.142	142	50.2
Carassius auratus	Goldfish	Freshwater	Water column	43	2.16	2.180	0.067	151	23.8
Leucisus idus melanotus	Golden orfe	Freshwater	Water column	27	2.34	2.183	0.078	152	28.1
Xenopus laevis	South African clawed frog	Freshwater	Water column	6	3.93	2.214	0.160	164	66.9
Alburnus alburnus	Bleak	Freshwater		7	3.59	2.234	0.147	171	63.3
Nitocra spinipes	Copepod	Freshwater		6	3.93	2.256	0.158	180	72.6
Neanthes arenaceodentata	Annelid worn	Salt water	Infauna	4	5.49	2.260	0.202	182	99.8
Tanytarsus dissimilis	Midge	Freshwater	Infauna	10	3.06	2.264	0.132	184	59.9
Artemia salina nauplii	Brine shrimp	Salt water		33	2.25	2.288	0.079	194	36.2
Lymnaea stagnalis	Snail	Freshwater		5	4.47	2.288	0.174	194	87.9
Gambusia affinis	Mosquito fish	Freshwater		8	3.37	2.310	0.147	204	75.4
Hydra oligactis	Brown hydra	Freshwater		5	4.47	2.329	0.174	213	96.6
Culex pipiens	House mosquito	Not applicable		5	4.47	2.333	0.174	215	97.5
Scenedesmus subspicatus	Green algae	Freshwater		24	2.41	2.345	0.086	221	45.2
Ambystoma mexicanum	Mexican axolotl	Freshwater		5	4.47	2.388	0.174	244	111
Daphnia cucullata	Cladoceran	Freshwater	Water column	5	4.47	2.394	0.174	248	112
Poecilia reticulata	Guppy	Freshwater		14	2.74	2.402	0.108	252	65.8
Aedes aegypti	Yellow fever mosquito	Not applicable		5	4.47	2.415	0.174	260	118
Tetrahymena elliottii	Ciliate	Freshwater		10	3.06	2.435	0.129	272	86.5
Menidia beryllina	Inland silverside	Salt water	Water column	8	3.37	2.465	0.143	292	104
Chlamydomonas angulosa	Green algae	Freshwater		29	2.30	2.524	0.083	334	65.7
Chlorella vulgaris	Green algae	Freshwater		34	2.22	2.650	0.078	447	82.3
Ankistrodesmus falcatus	Green algae	Freshwater		9	3.19	2.699	0.139	500	173
Chemical class corrections									
Aliphatic correction						0.000			
Alcohol correction						0.000			
Ketone correction						0.000			
Ether correction						0.000			
Halogenated chemical correction						−0.339	0.032	0.458	0.034
Polycyclic aromatic hydrocarbon correction						−0.352	0.053	0.445	0.055
Monoaromatic hydrocarbon correction						−0.109	0.034	0.778	0.061
Slope						−0.936	0.015		

[a] k_Z = extrapolation constants for use in HC5/HC95 calculations [10], where HC5 is the 5% hazard concentration and HC95 is the 95% hazard concentration.

[b] C_L^* = *log critical body burden in the target lipid of an organism.*

[c] Standard error (SE) is based on assumption that the estimation errors are gaussian. See Di Toro et al. [1] for formulas.

APPENDIX 2. Chemicals and their properties (at 25°C) measured in various data sets

	log(K_{ow})[a]	Molecular weight (g/mol)	Solid solubility (mg/L)[b]	Subcooled liquid solubility (mg/L)[b]
Ethane	1.730	30.00	1,350,000	1,350,000
Benzene	1.943	78.11	1,780,000	1,780,000
Propane	2.370	44.09	248,000	248,000
Toluene	2.438	92.14	515,000	515,000
Butane	2.868	58.12	61,400	61,400
Isobutane	2.869	58.12	48,900	48,900
o-Xylene	2.946	106.17	220,000	220,000
Cyclopentane	2.991	70.00	156,000	156,000
Ethylbenzene	3.006	106.17	152,000	152000
m-Xylene	3.032	106.17	160,000	160,000
p-Xylene	3.051	106.17	215,000	215,000
9,10-Anthracenedione	3.080	208.22	116,000	130,000
Naphthalene	3.256	128.19	31,000	110,000
Isopentane	3.335	72.15	13,800	13,800
Acenaphthylene	3.436	152.20	16,100	75,000
C3-Benzenes[c]	3.455	120.00	19,700	19,700
Pentane	3.471	72.15	38,500	38,500
9-Fluorenone	3.510	180.20	25,000	48,000
Methylcyclopentane	3.571	84.00	42,000	42,000
1-Methylnaphthalene	3.781	142.20	28,000	28,000
C1-Naphthalenes[c]	3.788	142.20	7,900	22,000
2-Methylnaphthalene	3.789	142.20	25,000	31,100
Acenaphthene	3.878	154.21	3,800	19,200
Fluorene	3.930	166.20	1,900	15,100
Biphenyl	3.936	154.21	7,000	20,000
2-Chloronaphthalene	3.940	162.64	5,500	18,000
1-Chloronaphthalene	3.950	162.64	5,400	5,400
Methylcyclohexane	3.963	98.19	14,000	14,000
Hexane	4.053	86.00	9,500	9,500
C2-Naphthalenes[c]	4.244	156.23	1,970	9,400
1,3-Dimethylnaphthalene	4.257	156.23	8,000	8,000
Dibenzothiophene	4.341	184.26	1,700	9,100
1-Methylfluorene	4.370	180.25	1,090	4,270
C1-Fluorenes[c]	4.370	180.25	1,510	8,400
Anthracene	4.546	178.20	45	3,500
2,3,5-Trimethylnaphthalene	4.570	170.20	580	5,200
2,3,6-Trimethylnaphthalene	4.570	170.20	740	5,200
Phenanthrene	4.584	178.23	1,100	6,210
n-Heptane	4.584	100.20	2,930	2,930
Dimethylbiphenyl[c]	4.692	182.00	530	4,400
C3-Naphthalenes[c]	4.730	170.25	440	3,800
C2-Fluorenes[c]	4.819	194.27	380	3,600
C1-Dibenzothiophene[c]	4.859	198.30	340	3,400
9-Methylanthracene	4.996	192.26	261	945
1-Methylphenanthrene	5.036	192.26	270	2,520
C1-Phenanthrene/anthracene[c]	5.037	192.26	180	2,300
2-Methylphenanthrene	5.040	192.26	180	2,300
Pyrene	5.126	202.26	132	2,610
Fluoranthene	5.190	202.26	260	1,700
C4-Naphthalenes[c]	5.220	184.28	97	1,500
C1-Fluoranthene/pyrene[c]	5.257	216.28	101	1,600
C3-Fluorenes[c]	5.318	208.30	80	1,400
C2-Dibenzothiophene[c]	5.332	212.30	78	1,400
3,6-Dimethylphenanthrene	5.340	206.29	73	1,300
4,6-Dimethyldibenzothiophene	5.450	212.30	53	1,100
C2-Phenanthrene/anthracene[c]	5.455	206.29	51	1,000
C2-Fluoranthene/pyrene[c]	5.557	230.31	40	950
Triphenylene	5.630	228.30	43	2,260
Benzo[*a*]anthracene	5.744	228.29	11	240
Chrysene	5.782	228.29	2.0	376
C3-Dibenzothiophene[c]	5.810	226.30	17	560
C4-Phenanthrene/anthracene[c]	6.357	234.34	3.0	190
Benzo[*b*]fluoranthene	6.341	252.32	1.5	38.9
C3-Fluoranthene/pyrene[c]	6.384	244.34	2.9	190
Benzo[*k*]fluoranthene	6.400	252.32	0.80	63.6
Benzo[*a*]pyrene	6.409	252.31	3.8	116
7,12-Dimethylbenzo[*a*]anthracene	6.420	256.35	50.00	455
Benzo[*e*]pyrene	6.447	252.30	4.0	130
Perylene	6.447	252.31	0.40	124
C2-Chrysenes/benzo[*a*]anthracene[c]	6.593	256.34	1.5	130
C4-Fluoranthene/pyrene[c]	6.687	258.35	1.1	110
C5-Phenanthrene/antracene[c]	6.700	248.37	1.1	99.5

APPENDIX 2. Continued

	$\log(K_{OW})$[a]	Molecular weight (g/mol)	Solid solubility (mg/L)[b]	Subcooled liquid solubility (mg/L)[b]
C1-Benzofluoranthene[c]	6.743	266.11	1.0	97.6
Benzo[*ghi*]perylene	6.886	276.34	0.26	83.2
C3-Chrysenes/benzo[*a*]anthracene[c]	6.972	270.36	0.47	62
Dibenz[*a,h*]anthracene	7.129	278.35	0.60	148
C2-Benzofluoranthene[c]	7.200	280.13	0.23	40.5
C4-Chrysenes/benzo[*a*]anthracene[c]	7.421	284.38	0.12	26

[a] log octanol–water partition coefficient (K_{OW}) computed from SPARC (Sparc Performs Automated Reasoning in Chemistry) [8].
[b] Solubility data from Mackay et al. [16–19] or computed from the following relationships: Solid solubility computed from $\log(S) = -1.4136 \log(K_{OW}) + 7.1022$, where solubility has units of mmol/L; subcooled solubility computed from $\log(S) = -0.8857 \log(K_{OW}) + 5.5367$, where solubility has units of mmol/L; for compounds that are liquid at room temperature, the solid solubility and subcooled solubility are equal.
[c] Mixture of isomers.