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Environmental Toxicology and Chemistry, Vol. 20, No. 9, pp. 2102–2113, 2001
Printed in the USA
0730-7268/01 $9.00 + .00

Hazard/Risk Assessment

APPLICATION OF A ΣPOLYCYCLIC AROMATIC HYDROCARBON MODEL AND A LOGISTIC REGRESSION MODEL TO SEDIMENT TOXICITY DATA BASED ON A SPECIES-SPECIFIC, WATER-ONLY LC50 TOXIC UNIT FOR *HYALELLA AZTECA*

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(*Received* 22 *August* 2000; *Accepted* 10 *January* 2001)

Abstract—Two models, a Σpolycyclic aromatic hydrocarbon (PAH) model based on equilibrium partitioning theory and a logistic-regression model, were developed and evaluated to predict sediment-associated PAH toxicity to *Hyalella azteca.* A ΣPAH model was applied to freshwater sediments. This study is the first attempt to use a ΣPAH model based on water-only, median lethal concentration (LC50) toxic unit (TU) values for sediment-associated PAH mixtures and its application to freshwater sediments. To predict the toxicity (i.e., mortality) from contaminated sediments to *H. azteca,* an interstitial water TU, calculated as the ambient interstitial water concentration divided by the water-only LC50 in which the interstitial water concentrations were predicted by equilibrium partitioning theory, was used. Assuming additive toxicity for PAH, the sum of TUs was calculated to predict the total toxicity of PAH mixtures in sediments. The ΣPAH model was developed from 10- and 14-d *H. azteca* water-only LC50 values. To obtain estimates of LC50 values for a wide range of PAHs, a quantitative structure-activity relationship (QSAR) model (log LC50 − log K_{ow}) with a constant slope was derived using the time-variable LC50 values for four PAH congeners. The logistic-regression model was derived to assess the concentration–response relationship for field sediments, which showed that 1.3 (0.6–3.9) TU were required for a 50% probability that a sediment was toxic. The logistic-regression model reflects both the effects of co-occurring contaminants (i.e., nonmeasured PAH and unknown pollutants) and the overestimation of exposure to sediment-associated PAH. An apparent site-specific bioavailability limitation of sediment-associated PAH was found for a site contaminated by creosote. At this site, no toxic samples were less than 3.9 TU. Finally, the predictability of the ΣPAH model can be affected by species-specific responses (*Hyalella* vs *Rhepoxynius*); chemical specific (PAH vs DDT in *H. azteca*) biases, which are not incorporated in the equilibrium partitioning model; and the uncertainty from site-specific effects (creosote vs other sources of PAH contamination) on the bioavailability of sediment-associated PAH mixtures.

Keywords—ΣPAH model Toxic unit Equilibrium partitioning model Logistic-regression model *Hyalella azteca*

INTRODUCTION

Sediment-quality guidelines have traditionally been developed on a single-pollutant basis. However, most instances of contamination in sediments involve mixtures. Such mixtures can act or interact in various ways, which may affect the magnitude and nature of the toxic effects. For polycyclic aromatic hydrocarbons (PAHs), which are typical narcotic compounds, these mixture effects are additive [1–3]. Based on this additivity of toxicity for PAH mixtures, Swartz et al. [4] suggested a ΣPAH model to quantitatively predict the toxicity of PAH mixtures in field sediment to marine and estuarine amphipods.

The ΣPAH model of Swartz et al. was developed based on 10-d median lethal concentration (LC50) values for four marine amphipods with sensitivities to PAH that were assumed to be similar. This model also assumed that the interstitial water concentration reflected sediment exposure through the use of equilibrium partitioning (Eq-P) [5], and that toxicity of PAHs in a mixture are additive. The model first predicts the interstitial water LC50 values ($LC50_{iw}$) based on a log $LC50_{iw}$ − log K_{ow} relationship developed using the measured toxicity in spiked-sediment tests. For the 13 priority PAHs, the predicted $LC50_{iw}$ values were used to calculate toxic units (TUs) for individual PAHs in the interstitial water, the concentrations of which were estimated by the Eq-P model. The number of TUs for PAHs that likely were insoluble was limited by the water solubility of the compound. Finally, sediment toxicity of field sediments was predicted based on the sum of TU:

$$\Sigma TU_i = \Sigma(PAH_{iw}/LC50_{iw})_i$$

and compared with the observed sediment toxicity.

Recently, the ΣPAH model was applied to derive sediment-quality guidelines for PAH mixtures [6,7]. Often, sediment-quality guidelines for individual PAHs developed from statistical methods such as effect range–low/effect range–median [8] and threshold-effect level/probable-effect level [9] do not represent the effects of individual compounds by themselves but, rather, the effects of compounds in unknown mixtures of covarying compounds, the cumulative toxicity of which is sufficient to exert adverse effects [6]. In contrast, the ΣPAH model is not based on correlation of the chemical concentration and biological response but, instead, represents an evaluation of causality based on TUs [10,11].

For application of the ΣPAH model to derivation of sediment-quality guidelines, a species-specific quantitative structure-activity relationship (QSAR) model was needed. How-

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ever, the QSAR regression equation that Swartz et al. [4] used for the ΣPAH model put together the LC50 values for the four marine amphipods as follows:

$$\log \text{10-d LC50}_{iwjk} = -1.328 \log K_{owj} + 2.919 \quad (1)$$

where $LC50_{iwjk}$ (mmol/L) is the interstitial water LC50 of the individual PAH j for species k and K_{owj} is the octanol/water partition coefficient of the individual PAH j. Therefore, this QSAR equation could not reflect differences of species sensitivity. Because of the size of the data set ($n = 6$), additional data regarding interstitial water LC50 values are needed to improve the accuracy of the QSAR regression used in the ΣPAH model [6]. Some studies have been recently conducted by Swartz et al. [3], Boese et al. [12], and Ozretich et al. [13] to validate the ΣPAH model. These studies evaluated the predictability of this model using the QSAR equation for the marine amphipods (Eqn. 1).

In the cases of PAH mixtures spiked to sediments [3,12], the sum of TUs was either less than additive or nonadditive. However, additivity was not disproved due to inaccuracies in extrapolating the octanol/water (K_{ow}) LC50 QSAR. When Ozretich et al. [13] tested the model at a creosote-contaminated site in Elliott Bay, Washington, USA, the sum of TUs for 33 measured PAHs tended to overestimate the observed mortality, but it was more predictive when only the parent 13 priority PAHs were used. In contrast, Di Toro and McGrath [14] applied a species-specific QSAR model for *Rhepoxynius abronius* to the same *R. abronius* sediment toxicity data in Elliott Bay using all the PAH congeners and successfully predicted the toxicity of the field sediment within a factor of two.

The ΣPAH model of Swartz et al. [4] is based on interstitial water LC50 TU as predicted from the Eq-P theory in spiked-sediment exposures. Di Toro and McGrath [14] expanded the ΣPAH model using the species-specific, water-only LC50 to develop Eq-P sediment guidelines for PAH mixtures. This approach is based on the Eq-P theory that interstitial water LC50 values are the same as water-only LC50 values [15]. To account for variations in toxicity due to differing species sensitivities, Di Toro and McGrath [14] used the target lipid model [15] as the species-specific QSAR model between water-only LC50 values and K_{ow}:

$$\log LC50_{jk} \cong \log CBR_k - m_N \log K_{owj} \quad (2)$$

where $LC50_{jk}$ (mmol/L) is the water-only LC50 of compound j for species k; CBR_k (mmol/kg octanol) is the constant critical body residue (CBR) for individual PAHs in species k, which is the internal tissue concentration corresponding to the $LC50_{jk}$; and m_N is the common narcosis slope for all species. Finally, sediment-quality guidelines for PAH mixtures could be established using the CBR_{5th} values that correspond with protecting 95% of aquatic species.

However, this approach [14] has not been fully validated by field toxicity data using a variety of test animals. The only field validation was conducted with *R. abronius* [14]. It is notable that this approach was not developed to use the measured interstitial water LC50 values from spiked-sediment toxicity tests but, rather, that it applies the species-specific, water-only LC50 database for derivation of sediment-quality guidelines.

This study applied the ΣPAH model based on water-only LC50 TUs to freshwater sediments for the prediction of sediment-associated PAH toxicity to *Hyalella azteca*, an organism broadly used for freshwater sediment toxicity testing. Our approach differed from the ΣPAH model of Swartz et al. [4] in that it did not use the interstitial water LC50 values from mixed species but, rather, used the species-specific, water-only LC50 values. To our knowledge, this is the first study to examine the uncertainty of the species-specific QSAR/Eq-P model for prediction of the toxicity of sediment-associated PAH mixtures. Because insufficient data regarding spiked-sediment toxicity tests with *H. azteca* are currently available to evaluate the concentration–response relationship in sediment exposure for PAH and to verify the ΣPAH model, a sum of TU–response logistic probability model from a field sediment toxicity database using *H. azteca* was developed based on co-occurrence analysis. Because the water-only LC50 TU is applied to field-collected sediment toxicity data without confirmation of sediment LC50 values in spiked-sediment toxicity tests, two potential complications were unavoidable. First, the bioavailability for sediment exposures was assumed to be driven by the organic carbon, according to the Eq-P theory. Second, there could well be contributions to observed toxic response due to unknown pollutants, including nonmeasured PAH in the sediment. Finally, in this study, the predictive reliability of the ΣPAH model based on Eq-P theory is assessed for the species-, chemical-, and contamination source–specific uncertainty.

MATERIALS AND METHODS

Water-only toxicity tests

For naphthalene, fluorene, phenanthrene, and pyrene, 10- and 14-d water-only toxicity tests were conducted using static-renewal exposure. The [^{14}C]-labeled PAHs were purchased from Sigma Chemical Company (St. Louis, MO, USA). Unlabeled PAHs were obtained from Aldrich Chemical Company (Milwaukee, WI, USA). The [^{14}C]-labeled PAHs were tested for purity before use by thin-layer chromatography (TLC) on silica plates (Alltech Associates, Deerfield, IL, USA) using hexane:benzene (8:2, v/v). Compounds less than 98% pure were purified by TLC. Stock solutions were generated by making a solution containing known amounts of nonlabeled and labeled PAH. New specific activities were determined for the isotope dilution. Concentrations of spiked test solutions were determined by liquid scintillation counting (LSC) on a Tri-Carb Liquid Scintillation Analyzer (Model 2500 TR; Packard Instrument, Meridien, CT, USA). Samples were corrected for quench using the external standards ratio method after subtracting background. The nominal specific radioactivity (μCi [^{14}C]-PAH added/μmol total PAH added) for each test solution was used to calculate the concentration of total PAH.

Huron River, Michigan, USA, water (total hardness: 165 mg/L as calcium carbonate; total alkalinity: 250 mg/L as calcium carbonate; pH 8.2) was used for toxicity testing. Test solutions were prepared by adding appropriate amounts of the (acetone-based) stock solution to filtered water (glass microfiber filters 934-AH; Whatman, Clifton, NJ, USA). Control test solutions were prepared with similar amounts of acetone (<0.5 ml/L).

Hyalella azteca originated from the National Biological Survey, Columbia, Missouri, USA. This stock was reared in a 37-L glass aquarium in filtered Huron River water under fluorescent light with a light:dark photoperiod of 16:8 h at a constant temperature of 23°C. A flow of approximately 10 L/d was passed through the aquarium. Animals were fed 0.25 g of ground TetraMin® fish food (TetraWerke, Melle, Germany) twice a week. Coiled plastic (coarse, washable filter media; Aquatic Ecosystems, Apopka, FL, USA) was used as a sub-

strate. Animals used in toxicity tests passed through a 1-mm mesh and were retained in a 0.5-mm mesh (two to three weeks old).

Twenty amphipods were exposed at 23°C to test solutions (200 ml in a 400-ml beaker) containing 1 cm^2 of sterile surgical gauze for substrate that was presoaked for 48 h in filtered Huron River water. The amphipods were fed 0.5 ml of yeast-cerophyl-trout chow per beaker every other day. Test waters in replicate beakers (three beakers/treatment) were renewed every day, and water samples (2 ml) were measured by LSC before and after renewing the test water. The numbers of dead and live animals were recorded daily, and dead animals were removed daily. The experiments were conducted with four or five treatments and three replicates per treatment. In each experiment, LC50 values were determined at each exposure time by trimmed Spearman-Karber method [16].

Prediction of LC50 values

To assess the total toxicity of multiple PAHs in sediment, the differences of the toxic potential of individual PAHs to test animals had to be addressed. We assumed that water-only LC50 values, the exposure time of which is the same as that in the sediment toxicity test, would reflect the sediment toxicity among individual PAHs based on the interstitial water concentration. First, LC50 values were determined for the selected PAH with the range of log K_{ow} values as wide as possible (3.37–5.18). However, it is impossible to determine the LC50 values for a high-molecular-weight PAH (HPAH), because the solubility limits of these compounds are lower than the predicted LC50 values. Using the measured LC50 values, a log LC50 − log K_{ow} regression equation was developed, and LC50 values of the other PAHs were predicted by this QSAR. These predicted values were then used for the calculation of TUs to predict the mixture effect of PAHs in field sediments.

A QSAR model for the prediction of LC50 values for each PAH was developed for multiple exposure times:

$$\log \mathrm{LC50}_{ij} = m_i \log K_{owj} + a_i \quad (3)$$

where $\mathrm{LC50}_{ij}$ is the median lethal concentration (mmol/L) at time i for compound j, a_i and m_i are the respective intercept and slope at exposure time i, and K_{owj} is octanol/water partition coefficient.

Calculation of TUs based on water-only LC50 values

A TU for sediments is defined as a dimensionless ratio between the environmental concentration with the same units, the interstitial water concentration (PAH_{iw}), and the LC50 for each PAH:

$$\mathrm{TU} = PAH_{iw}/\mathrm{LC50} \quad (4)$$

The PAH_{iw} was predicted from PAH concentrations in sediment (PAH_{sed}) and a fraction of organic carbon in sediment (f_{oc}) by the Eq-P model [5]:

$$PAH_{iw} = PAH_{sed}/(f_{oc} \cdot K_{oc}) \quad (5)$$

where K_{oc} (L/kg) is the sediment/organic carbon partition coefficient. Because the PAH_{iw} must not be higher than its solubility limit, for some PAH, the maximum number of TUs was limited by the solubility limit of the individual PAH. Finally, assuming the additivity hypothesis of PAH toxicity, the sum of TUs was calculated by adding the TUs for each PAH:

$$\sum TU_j = \sum (PAH^*_{iw}/\mathrm{LC50})_j \quad (6)$$

$$PAH^*_{iwj} = \begin{cases} PAH_{iwj} & \text{if } S_j \geq PAH_{iwj} \\ S_j & \text{if } S_j < PAH_{iwj} \end{cases} \quad (7)$$

where PAH^*_{iwj} is a solubility-corrected PAH_{iwj} and S_j is the solubility limit for compound j.

Development of a logistic-regression model using field sediment samples

A concentration–response model was developed using a *H. azteca* sediment toxicity (i.e., mortality) database containing 353 samples of 10- and 14-d *H. azteca* sediment toxicity test results with their corresponding chemistry data. All databases are subject to change over time, and this study employed the version of the database available in January 2000. The database was created as a co-operative effort involving the National Oceanic and Atmospheric Administration, U.S. Environmental Protection Agency (EPA), U.S. Geological Society, Macdonald Environmental Sciences, Environment Canada, and the States of Minnesota and Washington. The database included concentrations of metals, PAHs, polychlorinated biphenyls (PCBs), DDT, dichlorodiphenyldichloroethylene, and total organic carbon. The method used to screen and analyze the database for derivation of a logistic concentration–response relationship followed that described by Field et al. [17]. In brief, data were obtained from a variety of sources and followed the standard protocols for sediment chemistry and toxicity testing developed for each program. Individual samples were designated as either toxic or nontoxic according to the results reported by the investigators in the original study. This was usually based on a statistical comparison to a negative control. Toxic effects were reported in 62 (18%) of the samples.

The database was screened using an approach similar to that described by Ingersoll et al. [18]. Each toxic sample was classified as either an effect or no concordance, depending on whether the ratio of the concentration (expressed as the sum of TUs in the toxic sample to the mean number of the sum of TUs in the nontoxic samples collected in the same study area) was greater than two (i.e., effect) or was two or less (i.e., no concordance) instead of greater than one [8]. The samples coded as no concordance were excluded in the development of the logistic-regression models. After screening the original database, 322 samples remained, of which 31 were toxic.

Logistic-regression analysis was conducted to derive the concentration–response relationship, in which logit-transformed probability was related to the log-transformed sum of TUs:

$$\mathrm{logit}\ P = \log_e P/(1 - P) = \alpha + \beta X \quad (8)$$

$$P = \frac{\exp(\alpha + \beta X)}{1 + \exp(\alpha + \beta X)} \quad (9)$$

where P is the probability of observing a toxic effect, α is the intercept parameter, β is the slope parameter, and X is log(sum of TUs). The logistic model was applied to individual sample data. The probability of observing a toxic response was developed through an iteration procedure by fitting to the maximum-likelihood function in SYSTAT® (version 8.0; SPSS Institute, Evanston, IL, USA).

Statistics

Analyses of LC50 values were performed with TOXSTAT® (version 3.5; West EcoSystems Technology, Cheyenne, WY,

Table 1. Time-dependent median lethal concentration (LC50[*t*]) values for naphthalene, fluorene, phenanthrene, and pyrene at multiple exposure times[a]

Day	Naphthalene	Fluorene I	Fluorene II	Phenanthrene I	Phenanthrene II	Pyrene
1	—	—	—	3.48	—	—
2	—	—	—	2.86	—	—
3	29.0	5.48	—	1.60	—	—
4	27.3	4.25	3.35	1.46	—	—
5	25.3	3.87	3.06	1.42	—	0.480
6	24.6	3.73	2.95	1.42	—	0.460
7	23.9	3.52	2.88	1.43	—	0.445
8	23.1	3.47	2.88	1.43	—	0.430
9	21.9	3.30	2.81	1.36	1.34	0.405
10	21.2	3.16	2.72	1.30	1.32	0.381
11	19.9	—	2.57	—	1.30	0.376
12	19.2	—	2.56	—	1.28	0.341
13	17.9	—	2.55	—	1.27	0.316
14	16.6	—	2.43	—	1.26	0.297
15	—	—	2.23	—	1.24	0.292
16	—	—	2.20	—	1.21	0.272

[a] The LC50(*t*) values were calculated by trimmed Spearman-Karber (μmol/L). I an II indicate different sets of experiments.

USA). All statistical analyses were conducted using SYSTAT 8.0. The homogeneity among slopes (m_i) in the QSAR regression equation (Eqn. 3) was examined using the general linear model. If a significant interaction term is found between exposure time (*i*) and the octanol/water partition coefficient (*j*), the slopes (m_i) are significantly different from each other [19]. The intercepts (a_i) in the QSAR regression equations for the multiple exposure times were compared using analysis of covariance to evaluate the effect of exposure time and log K_{ow}. Finally, the common slope and the intercepts were concurrently estimated according to Equation 3 using MicroMath Scientist® (version 4.10; MicroMath Scientific Software, Salt Lake City, UT, USA). For the logistic-regression model, the slope, intercept parameters, and chi-square statistic were determined using the maximum-likelihood approach. Quantile confidence interval of the sum of TUs at a specific probability level was calculated using Fieller bounds [20].

RESULTS

Prediction of LC50 values

For naphthalene, fluorene, phenanthrene, and pyrene, LC50 values were determined for 10- or 14-d, water-only exposures (Table 1). At each test, LC50 values were calculated at multiple exposure times. To predict LC50 values for other PAH congeners, a QSAR regression equation (log LC50 vs log K_{ow}; Eqn. 3) was derived using a total of 55 LC50 values for the four chemicals. Slopes (m_i) of the QSAR equations at the various times were not significantly different ($p = 0.427$), but the exposure time significantly affected the $LC50_{ij}$ values ($p < 0.0005$). So, a simplified regression model was developed using a common slope (*m*) following the form of Equation 3. The estimated common slope was −0.969 (standard error, ±0.012), and its 95% confidence interval ranged from −0.992 to −0.946. Intercepts (a_i) in Equation 3 continuously decreased with increasing exposure times from 4 to 14 d. The use of only four relatively water-soluble PAHs to develop the QSAR reflects the solubility limits for producing acute toxicity with a single PAH through aqueous exposure. However, all PAH compounds were assumed to contribute to the toxicity of PAH mixtures by the same mechanism of action in proportion to the amount accumulated (see *Discussion*).

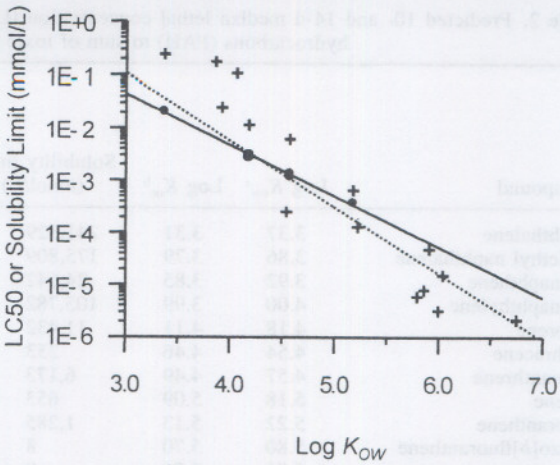

Fig. 1. Comparison of the quantitative structure-activity relationship equations for polycyclic aromatic hydrocarbons (PAHs) from *Hyalella azteca* (this study), marine amphipods [4], and target lipid model [15]. The measured 10-d median lethal concentration (LC50) values are used to predict LC50 values from K_{ow} for PAH compounds. The predicted LC50 values (● and solid line = *H. azteca*, dotted line = marine amphipods) for PAHs are compared with their solubility limit (+) in deionized water. Regression line of the target lipid model overlaps that of *H. azteca*; thus, the two regression lines are indistinguishable.

Using the common slope (*m*) from the simplified regression model, 10- and 14-d LC50 values (mmol/L) were estimated as follows ($r^2 = 0.993$):

$$\log(\text{10-d LC50}) = -0.969(\pm 0.012)\log K_{ow} + 1.543(\pm 0.060), \quad n = 6 \quad (10)$$

$$\log(\text{14-d LC50}) = -0.969(\pm 0.012)\log K_{ow} + 1.482(\pm 0.062), \quad n = 4 \quad (11)$$

The above 10-d LC50 equation was compared with QSAR regression equations for marine amphipods [4] and for a target lipid model using the common narcosis slope (−0.945 ± 0.014) [15] (Fig. 1). The slope for marine amphipods (−1.328 ± 0.138) was steeper than the slope for *H. azteca* (−0.969 ± 0.012). The slope for *H. azteca* was not significantly different from the common narcosis slope. For the models using *H. azteca* and the common narcosis slope, LC50 values estimated for HPAH are larger than their solubility limits. In contrast, for marine amphipods [4], most of HPAH LC50 values are lower than the respective solubility limits.

Calculation of TUs

The difference between the slopes of the models for the two amphipods (Fig. 1) results in variable contributions by individual PAHs to total toxicity (Table 2 and Fig. 2). The maximum number of TUs (TU limit) that can be contributed by individual PAH congeners was calculated by dividing the solubility limit by the LC50. The TU limits of individual PAHs for *H. azteca* and marine amphipods showed different patterns with increasing log K_{ow} (Fig. 2). In the case of *H. azteca*, its TU limit decreased with increasing molecular weight or log K_{ow}. For marine amphipods, TU limit was not strongly correlated with log K_{ow}. In addition, the toxicity of anthracene and HPAH (except fluoranthene and pyrene) were limited by their solubility to levels below their predicted LC50 values

Table 2. Predicted 10- and 14-d median lethal concentration (LC50) values and percentage of contribution for individual polycyclic aromatic hydrocarbons (PAH) to sum of toxic unit (TU) in *Hyalella azteca* and marine amphipods

Compound	Log K_{ow}[a]	Log K_{oc}[b]	Solubility limit[a] (nmol/L)	Predicted LC50 (nmol/L) *H. azteca*[c] 10-d LC50	*H. azteca*[c] 14-d LC50	Marine amphipods[d] (10-d LC50)	Max. TU at the solubility limit[e] *H. azteca*	Max. TU at the solubility limit[e] Marine amphipod
Naphthalene	3.37	3.31	241,829	19,042	16,546	27,303	12.7	8.9
2-Methyl naphthalene	3.86	3.79	175,809	6,336	5,506	—	27.7	—
Acenaphthene	3.92	3.85	24,642	5,544	4,818	6,290	4.4	3.9
Acenaphthylene	4.00	3.99	105,782	4,060	3,528	3,219	26.1	32.9
Fluorene	4.18	4.11	11,432	3,099	2,696	1,625	3.7	7.0
Anthracene	4.54	4.46	253	1,392	1,207	1,010	0.2	0.3
Phenanthrene	4.57	4.49	6,173	1,302	1,128	1,347	4.7	4.6
Pyrene	5.18	5.09	653	331	292	69	2.0	9.4
Fluoranthene	5.22	5.13	1,285	307	267	143	4.2	9.0
Benzo[*b*]fluoranthene	5.80	5.70	8	83	71	2	0.1	5.3
Chrysene	5.86	5.76	9	74	66	29	0.1	0.3
Benzo[*a*]anthracene	5.91	5.81	48	66	57	29	0.7	1.7
Benzo[*k*]fluoranthene	6.00	5.90	4	55	48	1	0.1	5.9
Benzo[*a*]pyrene	6.04	5.94	16	48	44	8	0.3	2.1
Dibenzo[*a,h*]anthracene	6.75	6.64	4	11	7	—	0.3	—

[a] [42].
[b] Log K_{oc} values were calculated as follows [5]: $\log k_{oc} = 0.00028 + 0.985 \log K_{ow}$.
[c] This study.
[d] [4].
[e] Max = maximum. Max. TU at the solubility limit is the maximum number of TUs when the ambient concentration reaches the solubility limit. Max. TU is calculated as follows: Max. TU = solubility limit (nmol/L)/LC50 (nmol/L).

(TU limit < 1). In contrast, for marine amphipods, only the toxicity of anthracene and chrysene were solubility limited below their LC50 levels. For *H. azteca* in field sediments, only anthracene, chrysene, and phenanthrene had sediment samples with predicted interstitial water concentrations greater than their solubility limits (for 12, 11, and 3 samples, respectively). For other PAHs, either no sample or only a single sample was above their respective solubility limits. The effect of solubility correction (Eqn. 6) was essentially not observed at less than 0.8 TU. At greater than 0.8 TU, the relative percentage decrease in TU generally was less than 20%, except that a 40% correction was observed in the most contaminated samples.

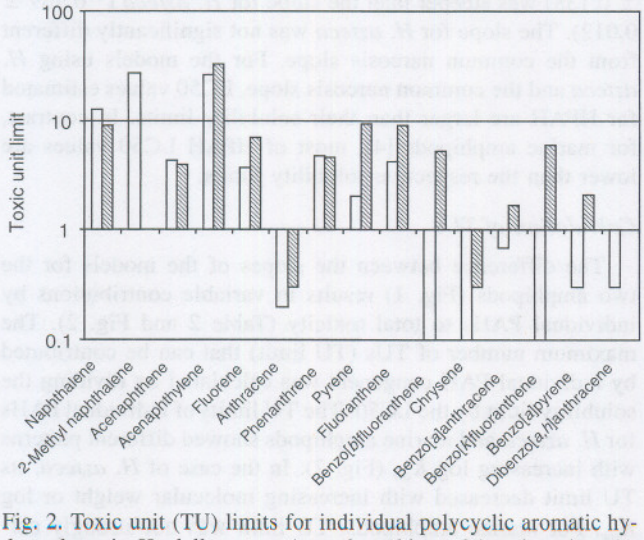

Fig. 2. Toxic unit (TU) limits for individual polycyclic aromatic hydrocarbons in *Hyalella azteca* (open bar; this study) and marine amphipods (closed bar) [4] in 10-d exposures. The TU limits were calculated by dividing the aqueous solubility limit by 10-d water-only median lethal concentration values.

The sum of TUs in the data set ranged from 0.00002 to 32 TU. The range of the sum of TUs in toxic samples (0.0001–32 TU) was similar to that in nontoxic samples (0.00002–3.9 TU) (Table 3). In nontoxic samples, the median value of the sum of TUs was 0.009 TU, and the 25th and 75th percentile values were 0.002 and 0.035, respectively. In toxic samples, the median value of the sum of TUs was 0.027 TU, and the 25th and 75th percentile values were 0.006 and 0.89 TU, respectively.

After screening the original 353 samples based on co-occurrence analysis, 31 toxic samples (0.00006–0.08 TU) were excluded from further analysis. The screening procedure reduced the variability in the observed probability for low TU numbers by removing sediment samples that were probably toxic for reasons other than PAH. Of the 322 samples re-

Table 3. Comparison of the distributions of concentration (sum of toxic units) of toxic and nontoxic samples within total and screened database

	Toxic samples in total database	Nontoxic samples in total and screened database	Toxic samples in screened database
n[a]	62	291	31
Minimum toxic unit	0.00056	0.00021	0.074
Maximum toxic unit	32	3.9	32
Percentiles	Concentrations (sum of toxic units)		
10th	0.0014	0.00060	0.011
15th	0.0037	0.0010	0.012
25th	0.0062	0.0021	0.092
50th	0.027	0.0094	0.92
75th	0.95	0.036	5.1
85th	2.7	0.081	15

[a] n = Number of samples.

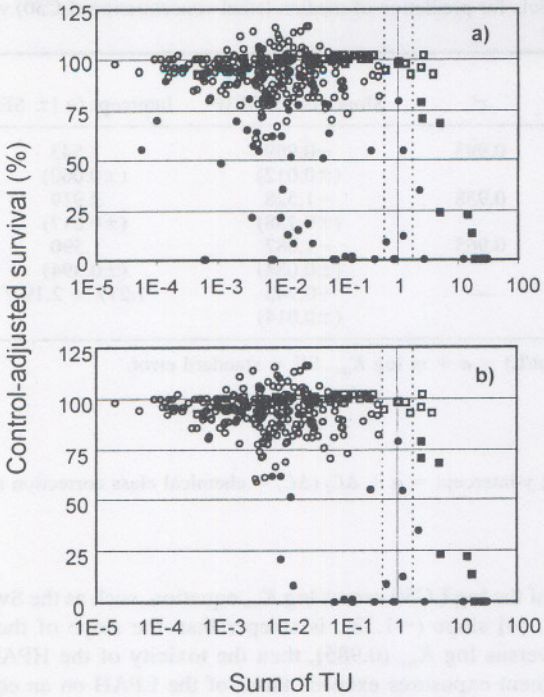

Fig. 3. Observed control-adjusted percentage survival versus the sum of toxic units (TU) in the *Hyalella azteca* sediment toxicity test database before screening (**a**) and after screening (**b**) the original database. Squares are samples from the McCormick and Baxter site; circles are samples from the other sites. Open markers and closed markers indicate nontoxic and toxic samples, respectively. Solid lines are the predicted number of TU corresponding to 50% probability of toxicity; dotted lines are uncertainty of a factor of two.

maining, 31 samples were toxic, and the sum of TU ranged from 0.007 to 32 TU (Table 3).

Within the screened database, 15 of the 22 samples with TU > 1 and all of the nontoxic samples with TU > 0.5 were from the McCormick and Baxter site (Willamette River, OR, USA) (Fig. 3). In samples from this site, the highest sum of TUs in nontoxic samples was 3.9 TU. In sites other than McCormick and Baxter, no nontoxic samples were greater than 0.5 TU.

Concentration–response relationship in the H. azteca *sediment toxicity database*

As shown in Figure 3a, no clear relationship was found between the sum of TUs and the control-adjusted percentage of survival, but at greater than 3.9 TU, all samples were toxic. At greater than 1 TU, the percentage survival ranged from 0 to 100%. Between 0.01 and 1 TU, most of the toxic samples showed survival below 20%. However, if the probability that a sample was toxic is considered as a response variable, a significant dose–response relationship between the sum of TUs and the probability was developed by a logistic-regression analysis (Table 4).

The logistic regression relationship between the log sum of TU and probability that a sample was toxic yielded a chi-square statistic of 76.682 (n = 322, $p < 0.0005$) (Table 4). The intercept (α) in Equation 9 was −0.195 ± 0.306, which is not significantly different from zero (p = 0.524). The slope (β) in Equation 9 was 1.733 ± 0.257, which is significantly different from zero ($p < 0.0005$). The sum of TUs for a 50% probability that a sediment would be toxic was 1.3 TU (95% CI, 0.6~3.9 TU). The corresponding sum of TU for a 10% probability was 0.3 TU (95% CI, 0.1~0.5 TU). The higher the probability of toxicity, the wider the confidence intervals. For example, the sum of TUs to produce 90% probability of toxicity was 24 TU, and its 95% confidence interval ranged from 7 to 205 TU. The chi-square statistic normalized to the sample size as a measure of goodness-of-fit was 0.24, which is similar to those of individual PAH models as determined by Field et al. [17].

Table 4. Comparison of the results of logistic regression models (LRMs) with and without the McCormick and Baxter (M&B) site[a]

	LRM for screened database	
	With M&B site	Without M&B site
Number of samples (n)	322	265
Chi-square value	76.682	62.111
Chi-square value/n	0.24	0.23
p	<0.0005	<0.0005
Intercept α (±SE)[b]	−0.195 (±0.306)	0.094 (±0.511)
p	0.524	0.077
Slope β (±SE)[b]	1.733 (±0.257)	2.191 (±0.377)
p	<0.0005	<0.0005
T10 (95% CI)[c]	0.3 (0.1 ~ 0.5)	0.04 (0.02 ~ 0.07)
T50 (95% CI)[c]	1.3 (0.6 ~ 3.9)	0.4 (0.2 ~ 1.2)
T90 (95% CI)[c]	24 (7 ~ 205)	4 (1.3 ~ 33)

[a] The LRM was developed for the relationship between log sum of toxic unit and probability of toxicity for *Hyallela azteca* 10-d survival test.

[b] Intercept (α) and slope (β) were estimated by Equation 9. SE = standard error.

[c] T10, T50, and T90 are the concentrations that would give a response of 10%, 50%, and 90% of probability of toxic samples. CI = confidence interval.

An additional logistic-regression analysis was conducted for the data without inclusion of the samples from the McCormick and Baxter site (Table 4). This produced a significant relationship but shifted the sum of TUs to produce a 50% probability of toxicity to 0.4 TU (95% CI, 0.2~1.2 TU). This reduces the dose by approximately a factor of three for the median response compared to the model with the McCormick and Baxter site included.

DISCUSSION

Prediction of LC50 values based on a QSAR model

In this study, LC50 values at multiple exposure times were derived from Equation 3, which related log $LC50_{ij}$ (mmol/L) to log K_{owj}, where i is the time and j and is the compound. The slopes (m_i) among the various exposures times (Eqn. 3) are not significantly different. However, the intercepts (Eqn. 3; $a_i = \log CBR_i - a_i$) decrease with increasing exposure times ranging between 4 and 14 d, even though the internal PAH concentration reached steady state after 48-h exposure [21]. Although PAHs are a typical narcotic chemical class, it is necessary to include exposure time as an important variable in the QSAR model for prediction of the toxicity. This result is supported by measured CBR values for PAHs in *H. azteca,* which showed a time-dependent pattern [21].

For HPAH, the acute toxicity cannot usually be determined by aqueous exposures because of their solubility limits. Yet, HPAH are expected to contribute to the overall toxicity in an additive manner on an equimolar basis in *H. azteca,* as was observed for *Diporea* sp. [22]. Thus, predicted LC50 values for HPAH were extrapolated from LC50 values for a low-

Table 5. Comparison of quantitative structure-activity relationship (QSAR) models for prediction of median lethal concentration (LC50) values using K_{ow} values

QSAR models	No. species	No. chemicals	No. LC50 values	r^2	Slope (m [± SE])[a]	Intercept (a [± SE])[a]
10-d *H. azteca*[b]	1	4	55	0.993	−0.969 (±0.012)	1.543 (±0.060)
Sum of PAH[c]	4	3	6	0.958	−1.328 (±0.138)	2.919 (±0.617)
10-d *R. abronius*[d]	1	4	8	0.965	−1.087 (±0.084)	1.590 (±0.394)
TLM[e]	33	156	1,055	—	−0.945 (±0.014)	1.277 ~ 2.197[f]

[a] The slope and intercept in QSAR model equations as follows: log LC50 (mmol/L) = $a + m \log K_{ow}$. SE = standard error.
[b] This study.
[c] [4].
[d] A QSAR model equation recalculated from Swartz et al. [3].
[e] TLM-target lipid model [15].
[f] Range of the y-intercepts (a) corrected for PAH in the tested species (n = 33); y-intercept = $a + \Delta C_l$ (ΔC_l = chemical class correction to the y-intercept a). Range of the y-intercept = ((1.54–0.263), (2.46–0.263)).

molecular-weight PAH (LPAH) assuming the same toxic mechanism (i.e., narcosis). Meador et al. [23] demonstrated that deposit feeders and nondeposit feeders receive a large percentage of their body burden of two- and three-ring compounds (plus the four-ringed compounds pyrene and fluoranthene) through interstitial water, regardless of feeding mode, which would produce a similar pattern of lipid-normalized bioconcentration factors (BCFs; i.e., exposure) based on interstitial water concentration for infaunal species. However, for HPAH, BCF values of the nondeposit feeder *R. abronius* were lower than those values of the nonselective deposit feeder *Armandia brevis*. In the case of *R. abronius*, BCFs for HPAH were close to the predicted values based on the linear relationship between log K_{ow} and log BCF only when expressed in terms of the freely dissolved PAH concentration in interstitial water. Therefore, assuming similar bioaccumulation processes between *R. abronius* and *H. azteca*, bioaccumulation (exposure) and, therefore, LC50 values for HPAH can be predicted by a QSAR regression model (log LC50 − log K_{ow}) based on the same toxic mechanism for LPAH and HPAH.

A QSAR regression model is usually derived on the basis of individual species [1]. However, the QSAR model of Swartz et al. was derived using LC50 values (n = 6) of four marine amphipods assuming similar sensitivities. Thus, this QSAR model could not consider the differences in species-specific sensitivity. Therefore, we used interstitial water data from Swartz et al. [3,4] for *R. abronius* to recalculate the QSAR results in a slope similar to that for *H. azteca* (Table 5). This result suggests the potential for differential species sensitivity to PAH toxicity when compared to the original model of Swartz et al. [3,4]. From the recalculated regression, the predicted LC50 values for LPAH decreased by a factor of two, and those for HPAH increased by a factor of two. However, those for fluoranthene and pyrene did not change. When the recalculated QSAR model is compared with the model for *H. azteca*, *H. azteca* and *R. abronius* appear to be similarly sensitive to PAH.

Calculation of TUs and sediment effect concentration

The ΣPAH model is based on predicted LC50 values of individual PAHs. The predicted LC50 values and the other model outputs of the ΣPAH model are very sensitive to the slope of the QSAR regression equation (Eqn. 3). If the slope (m) of the log LC50 versus log K_{ow} equation, such as the Swartz et al. [4] slope (−1.33), is steeper than the slope of the log K_{oc} versus log K_{ow} (0.985), then the toxicity of the HPAH in sediment exposures exceeds those of the LPAH on an equivalent-concentration basis. Thus, if the slope (m + 0.985; Eqn. 12) for the concatenation of the relationships for log LC50 versus log K_{ow} and log K_{oc} versus log K_{ow} is less than zero, such as for marine amphipods, the organic carbon–normalized sediment LC50 ($LC50_{s,oc}$; mmol/kg organic carbon) decreases with increasing log K_{ow} (Fig. 4):

$$\log \text{10-d LC50}_{s,oc} = (m + 0.985) \log K_{ow} + a \quad (12)$$

In contrast, if the slope is greater than zero, as for *H. azteca*, the $LC50_{s,oc}$ increases with increasing K_{ow}. For *H. azteca*, the 10-d $LC50_{s,oc}$ can be calculated as follows:

$$\log \text{10-d LC50}_{s,oc} = 0.016 \log K_{ow} + 1.543 \quad (13)$$

Within the range of log K_{ow} from 3.4 to 6.8, sediment effect concentrations ($LC50_{s,oc}$) were predicted to be relatively constant (39–43 mmol/kg organic carbon). Depending on the

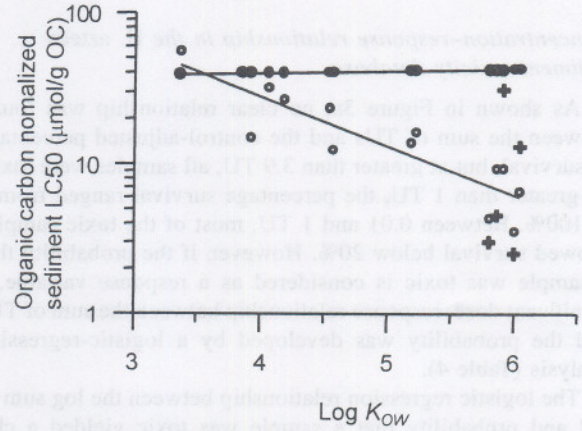

Fig. 4. Comparison of predicted 10-d organic carbon normalized sediment median lethal concentration (LC50) values for individual polycyclic aromatic hydrocarbon congeners between *Hyalella azteca* (●; this study) and marine amphipods (○) [4] and sediment concentration corresponding to aqueous interstitial water solubility limit (+). The organic carbon–normalized sediment LC50 values were calculated using Equation 12.

slope (m + 0.985; Eqn. 12), sediment effect concentrations for HPAH may be lower or higher than the sediment concentration corresponding to the solubility limit in interstitial water. As with the model for *H. azteca,* the QSAR model of Swartz et al. [4] estimated LC50 values of HPAH, except for fluoranthene, from a regression equation derived from LPAH LC50 values. Using the ΣPAH model for marine amphipods, approximately 86% of toxicity in field sediments apparently results from HPAH [4] due to the smaller estimated LC50 values for HPAH. In contrast, the relative contribution of HPAH to sum of TUs in field sediments for *H. azteca* was 60%. If pyrene and fluoranthene are excluded from HPAH, the relative contribution of HPAH to sum of TUs in field sediments was 58% and 19% in the model of Swartz et al. and the *H. azteca* model, respectively. Furthermore, when *R. abronius* were exposed to a mixture of HPAH at the empirically determined interstitial water solubility limit and 14.32 TU, no significant toxicity was observed. These results suggest that the QSAR model of Swartz et al. overestimated the toxicity of the HPAH compounds. This may be due to a 10-d exposure being insufficient for tissue residues to reach full potential for bioaccumulation [12]. However, for *H. azteca,* based on the relationship between log K_{ow} and log k_e [21], the predicted $t_{½}$ values for HPAH with log K_{ow} = 6 is 16 h, so tissue residue should easily reach steady state during a 10-d exposure. Whether this is sufficient for *R. abronius* is unknown, because the toxicokinetics for this species have not been determined.

Factors affecting the concentration–response relationship in the field-collected sediment toxicity database

The ΣPAH and logistic-regression models for PAH mixtures used field sediment toxicity data with matching chemistry data using TU based on water-only LC50 values. The approach used to evaluate the toxicity of PAHs in the field sediment samples makes the same assumptions as the U.S. EPA equilibrium sediment guidelines (ESGs) for a PAH mixture [24]. Those assumptions include that individual PAH congeners have additive toxicity, and that sediment interstitial water exposure can be predicted from Eq-P theory using water-only LC50 values. This approach (based on water-only LC50 TU) contrasts with the approach of Swartz et al., which is based on interstitial water LC50 values estimated by the Ep-P theory from spiked-sediment toxicity tests. Because the water-only LC50 TU is applied to sediment toxicity data without confirmation, two potential complications were unavoidable. First, bioavailability for sediment exposures was assumed to be driven by the organic carbon, according to the Eq-P theory. Second, there could well be contributions to the observed toxic response due to unknown pollutants, including nonmeasured PAH in the sediment.

Bioavailability in sediment exposure. To predict toxicity, the sum of TUs was used to develop the relationship between toxicity and the contamination level in field sediment. If no toxicity occurs from covarying compounds, a sum of TUs equal to one is expected to produce 50% mortality [14,24]. If covarying compounds that contribute to toxicity are present, the sum of TUs required to produce 50% mortality should be less than unity. However, if factors that affect bioavailability, such as the lack of Eq-P or the presence of a more highly sorptive phase, should occur, the sum of TUs corresponding to a 50% mortality would be greater than unity.

Considering a factor of two as the approximate uncertainty for Eq-P model–derived sediment organic carbon–normalized LC50s [25], no nontoxic sediments or sediments with less than 50% mortality at greater than 2 TU would be expected. In the opposite case, no toxic sediments showing more than 50% mortality at less than 0.5 TU would be expected. The *H. azteca* database contained three nontoxic sediment samples and three toxic sediment samples with less than 50% mortality at greater than 2 TU (Fig. 3). These six sediment samples represented approximately 40% of the samples greater than 2 TU, and all were collected from the McCormick and Baxter site. This apparent overestimation of toxicity by the TU model may be due to reduced bioavailability or differences in toxicity test conditions. According to Di Toro and McGrath [14], this result can be interpreted as a failure of the Eq-P model to predict the sediment toxicity for *H. azteca.* Spiked-sediment toxicity tests with *H. azteca* have produced similar observations (i.e., overestimation of toxicity) for PAHs [26–28] and dieldrin [29].

It is possible that the reduced response relative to the number of TU results from abiotic factors, such as the presence of a resistant PAH phase (e.g., coal or tar-like material) or a more highly sorptive phase (e.g., soot carbon) in field sediments [14], thus reducing exposure. However, in a spiked-sediment toxicity test using *H. azteca,* the response relative to the interstitial water concentration depended on details of exposure conditions such as intermittent flow-through (i.e., static renewal) and static exposures [26]. Greater responses for *H. azteca* were found at the same treatment levels for a HPAH mixture in static exposures compared to static-renewal exposures [28]. In a dieldrin-spiked sediment toxicity test using *H. azteca* with the flow-through exposure, the 10-d LC50 value based on the Eq-P model–predicted interstitial water concentration was, at a maximum, larger by a factor of 25 than the 10-d water-only LC50 [29]. Under static exposures, the overlying water LC50 values were more similar to the water-only LC50 values than LC50 values based on interstitial water [28]. With fluoranthene under static-renewal exposures, ratios of the estimated interstitial water LC50 value to the water-only LC50 value ranged from 3.2 to 6.2 [27,30,31]. Most sediment toxicity tests in the *H. azteca* database of field-collected samples used a static-exposure system. Therefore, the apparent overestimation of toxicity by the *H. azteca* ΣPAH model could have resulted from factors such as influence of avoidance behavior or presence of highly sorptive phases. In the few cases that used static renewal [29], abiotic factors, such as the dilution resulting from flushing of overlying water, may have been responsible for the lower-than-expected mortality.

In the *H. azteca* database, all of the nontoxic samples and half of the toxic samples greater than 1 TU were from one site contaminated by creosote (i.e., McCormick and Baxter site) (Fig. 3a). In samples from the McCormick and Baxter site, no toxic samples were less than 3.9 TU, the highest sum of TUs in nontoxic samples. In contrast, the highest sum of TUs in nontoxic samples from other sites was 0.5 TU. These results suggest that the ΣPAH model performs more as expected from Eq-P theory for *H. azteca* if samples from the McCormick and Baxter site are excluded. The creosote was assumed to exhibit normal partitioning behavior, as predicted from K_{ow}/K_{oc} relationships for other petrogenic sources [13,25]. In Elliot Bay, which is a marine site contaminated by creosote, the PAH partitioning appeared to be in equilibrium, and the site was suitable for application of the Swartz et al. ΣPAH model. However, in the McCormick and Baxter site, the data,

although sparse, suggest a site-specific limit to the bioavailability of sediment-associated PAHs [32].

Nonmeasured PAH issue in field sediments. Application of the ΣPAH model to field-collected sediments may also be affected by the presence of unmeasured PAHs contributing to the toxicity. Based on analysis of field-collected sediments, Di Toro and McGrath [14] estimated that the sum of TUs in field sediments would be underestimated by approximately a factor of three (90% CI, 2~12) if only 13 PAHs are accounted for. However, accounting for unmeasured PAHs in this data set would only increase the overestimation of toxicity. Thus, before attempting to account for potential unmeasured PAHs, the apparent bioavailability limitation of *H. azteca* to sediment-associated contaminants must be accounted for. The impact of the actual exposure conditions to sediment-associated PAHs in the sediment toxicity bioassay cannot be estimated separately from the influence of unknown contaminants. These factors can only be separated in well-designed, laboratory spiked-sediment bioassays or by measuring the exposure in organisms.

For this data set, insufficient data were available to estimate the impact of the actual PAH exposure to the relative contribution of nonmeasured PAHs in the toxicity response. In contrast with our model, the model of Swartz et al. may have minimized the effect of bioavailability limitations. This results from the use of interstitial water LC50 values from sediment exposures to develop the QSAR for comparison with field sediment interstitial water concentrations in which both were estimated from Eq-P theory. Thus, in the ΣPAH model of Swartz et al., it may be possible to separate the effect of nonmeasured PAHs on the toxicity response in field sediment by comparing the concentration–response relationships between laboratory spiked and field sediments having complex mixture of pollutants.

The ΣPAH model of Swartz et al. included a concentration–response model derived from single PAH spiked-sediment toxicity data. The sum of TUs corresponding to a 50% probability of toxicity in PAH-spiked sediment was 0.725 TU [4]. This value is smaller than unity, because it is not based on percentage mortality but, rather, on the probability of toxicity (>24% mortality for each species). However, the sum of TUs corresponding to a 50% probability of toxicity in sites where PAHs are a primary contaminant, in sites where PAHs are not a primary contaminant, and in total field sediments were 0.3, 0.1, and 0.2 TU, respectively (Fig. 5). (These values were recalculated from numbers provided by Tables 4 through 6 in Swartz et al. [4].) These data and the Elliot Bay data set [13] were re-evaluated by Di Toro and McGrath [14], and the toxicity of sediment-associated PAHs was found to be severely underestimated if alkylated PAHs were not considered. Therefore, in the case of the ΣPAH model of Swartz et al., the frequency of toxicity for PAH in field sediments can be underestimated, on average, by a factor of three, because the sum of TUs was calculated using only 13 PAHs.

Recently, Ozretich et al. [13] reported that TUs calculated from the Swartz et al. ΣPAH model using 33 PAHs tended to overestimate the observed mortality at the Elliot Bay site. They concluded that the toxicity was dominated by the parent PAH, with log $K_{ow} < 6.0$, and that the alkylated PAHs did not contribute significantly [13]. In contrast, Di Toro and McGrath [14] successfully validated an Eq-P model using the same toxicity data from Elliot Bay within an uncertainty factor of two. This model used the common narcosis slope (−0.969 ± 0.012) instead of that determined by Swartz et al. (−1.328 ± 0.138) for prediction of LC50 values [15]. Based on the two analyses, which both used an additive model for PAH toxicity, the contribution of alkylated PAHs to the toxicity in the field depends on which model is accepted.

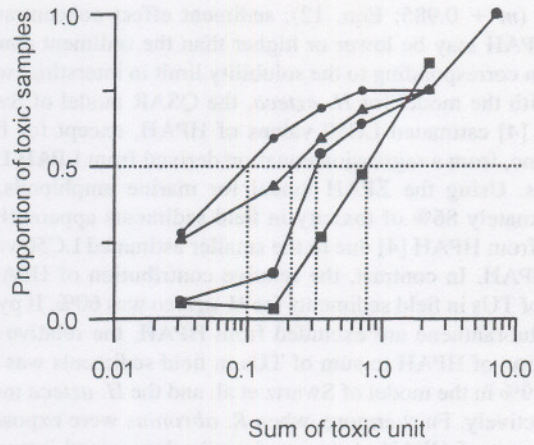

Fig. 5. Recalculation of the proportion of toxic samples in a 10-d *Rhepoxynius abronius* sediment toxicity test for polycyclic aromatic hydrocarbon (PAH)–spiked sediment and field sediments [4]. Dotted lines show the sum of toxic units (TUs) corresponding to a 50% probability of toxicity in each sediment sample group. The 50% probability of toxicity in PAH-spiked sediment (■) was 0.725 TU. In the case of field sediments, however, this was 0.3 TU (●; field sediments in which PAH is the primary contaminant), 0.1 TU (○; field sediments in which PAH is not the primary contaminant), and 0.2 TU (△ total field sediments).

The above results suggest that the effects of nonmeasured PAHs, such as alkylated PAHs, in field sediments need to be considered a potentially significant factor affecting the concentration–response relationship. If we assume a factor of 2.78 as a conversion factor of toxicity from 13 to 37 PAHs [14], then in the logistic-regression model, 3.6 TU (1.3 TU × 2.78 ≅ 3.6) would be required to produce a 50% probability of toxicity. In the case of the ΣPAH model, 1 TU is expected to yield 50% mortality; adjusting for this conversion factor, then the sediments in Figure 3b with 0.36 TU (0.36 TU × 2.78 = 1 TU) would be expected to exhibit 50% mortality. However, many of the sediment samples ($n > 20$) at or above this value are not toxic or show less than 50% mortality. Thus, accounting for alkylated PAHs would result in significant overprediction of toxicity. This further suggests that the PAHs may be less bioavailable than predicted by the ΣPAH model, or that differences in the toxicity test conditions affected the toxicity.

Logistic-regression model based on the sum of TUs

In contrast with the Eq-P theory–based ΣPAH model, the logistic-regression model showed an apparent relationship between the sum of TUs and the probability of toxicity in field sediment with an uncertainty of ± a factor of 3 (Table 4). This logistic-regression model has a role similar to a concentration–response model in the ΣPAH model of Swartz et al. The logistic-regression model was based on the co-occurrence analysis for field sediment toxicity data matching chemistry data. To exclude toxic samples in which PAHs likely are not associated with the observed toxicity, toxic samples with PAH concentrations lower than twice the mean PAH concentration in nontoxic samples from the same site were removed from the logistic-regression analysis [8,17,18]. Through this screening procedure, the influence of other contaminants with a dis-

tribution pattern different from that of the PAHs could be reduced. However, the logistic-regression model can overestimate the effect of PAHs, because the screening procedure cannot remove the influence of covarying contaminants.

The logistic regression model was different depending on whether the McCormick and Baxter site was included. Excluding this site, the sum of TUs estimated to result in 50% probability of toxicity in the 10-d *H. azteca* test was 0.4 TU (95% CI, 0.2~1.2 TU); including this site, the value was 1.3 TU (95% CI, 0.6~3.9 TU) (Table 4). These results could reflect reduced bioavailability of PAHs at the McCormick and Baxter site from overestimation of exposure for sediment-associated PAHs or the contribution of covarying contaminants at the other sites. However, the extent of these contributing factors, which affect predicted values in opposite directions, cannot be resolved with the logistic-regression model. Thus, the logistic-regression model cannot be interpreted as a cause–effect model, because the actual level of PAH exposure was not confirmed.

To our knowledge, the only previous controlled studies exposing *H. azteca* to spiked-sediment tests used fluoranthene [27,31] and a mixture of HPAH [28]. In these experiments, the measured sediment LC50 values were at least a factor of three greater than the predicted sediment LC50 values using Eq-P theory and water-only LC50 values [27,28,31]. However, the lethal body residue for sediment exposure and water-only exposure were essentially the same [27,30,31]. Thus, it required more than 3 TU to produce the measured results in sediment exposures. These results are the only data available for determining the actual exposure conditions for *H. azteca* to sediment-associated PAHs.

Furthermore, without confirmation of the actual exposure for field sediments, the result of the logistic-regression model without the McCormick and Baxter site cannot be interpreted as a validation of the ΣPAH model relative to cause and effect, because the logistic model can reflect both the influence of overestimation of exposure and the impact of other pollutants in concert with PAHs on sediment toxicity. Using the *H. azteca* database to evaluate the performance of either the ΣPAH or logistic-regression models is hampered by the influence of a single site, which is the source for a large proportion of the samples with high PAH concentrations.

Meanwhile, logistic-regression models based on dry-weight concentrations for individual contaminants have been applied to a marine amphipod sediment toxicity database [17,33]. These models [17,33], as well as the model in this study, suggested that logistic regression could be used to estimate the percentage of samples expected to be toxic at various PAH concentrations in field sediments. In addition, a logistic-regression model is suitable for screening field sediments. It enables users to select the probability of effects that correspond to their specific assessment or management objective, and it can be used to estimate the probability and CI of observing specific biological effects at given PAH concentrations [33].

Limitations for the ΣPAH model

The Eq-P theory–based ΣPAH model was considered to be useful for developing a plausible concentration–response relationship and for quantitatively predicting the toxicity of sediment contaminated with a complex mixture of PAHs [34]. In addition, the ΣPAH model can be used to identify contaminants responsible for sediment toxicity when this model is based on independent evaluation of causality, such as a spiked-sediment toxicity test [6]. However, the sources of the uncertainty, such as species- and chemical-specific biases and site-specific effects (e.g., differences in contamination source and in the composition of sediment organic matter) in the bioavailability of sediment-associated PAH were not fully incorporated in the ΣPAH model based on water-only TU. This model only incorporated the variation in relative bioavailability due to changes in the amount of organic carbon in the sediment. These conditions are the same as those recommended by the U.S. EPA nonpolar organic compounds using Eq-P theory [24,25].

In contrast to spiked-sediment toxicity tests, field sediment toxicity tests reflect different sources of uncertainty, such as toxicity of the co-occurring contaminants, and other site-specific effects, such as the incorporation of PAHs in particle phases (e.g., soot). In both the ΣPAH and logistic-regression models, bioavailability of sediment-associated PAH is the common source of uncertainty. When PAH-spiked sediment toxicity tests were used for derivation of U.S. EPA ESGs for PAH mixtures, the average values for LC50 ratios, either the ratio of interstitial water LC50 to the water-only LC50 or the ratio of observed and predicted organic carbon–normalized sediment LC50, were 1.60 and 1.67, respectively [24]. However, these values were calculated using only data for marine amphipods such as *Eohaustorius estuaries, Leptocheirus plumulosus,* and *R. abronius.* Recently, *L. plumulosus* exposed to fluoranthene showed a ratio of more than 5.4 for 10-d LC50 compared to the water-only LC50 [35]. For *H. azteca,* ratios of 3.2 to 6.2 have been reported for fluoranthene [27,30,31]. These results suggest either a significant species-specific bias in the ΣPAH model or an overestimation of the bioavailability.

The ratio of measured and predicted sediment LC50 values in spiked-sediment tests are chemical specific as well as species specific. For *H. azteca* exposed to DDT, the ratio is approximately unity [36]. However, for fluoranthene, the ratio is more than three, even though the tests used the same exposure conditions, sediment source, and *H. azteca* culture. For endrin, organic carbon–normalized LC50 values decreased with increasing organic carbon content [37]. A simple Eq-P model based on K_{ow} and K_{oc} values cannot interpret these results. In a PAH-spiked sediment bioassay using *Diporea* sp., PAH bioaccumulation was kinetically limited by a slower desorption rate from sediment particles into interstitial water compared to the uptake rate by the organism [38]. When PCB bioavailability appears to be determined by the amount of organic carbon in sediment, the bioavailability (sediment uptake rate constant, or k_s) of sediment-spiked PAH using sediments from several sources was controlled by the polarity of organic matter represented by the C:N ratio [39]. Other studies have confirmed that the quality of organic matter (e.g., aromaticity [40] and nutritional character [41]) is critical for controlling bioavailability.

In the *H. azteca* database, site-specific uncertainty was greater than the expected uncertainty of an Eq-P theory–based model (± a factor of two). The sum of TUs corresponding to a 50% probability of toxicity without the McCormick and Baxter site (0.4 TU) was different by a factor of 10 compared to the same probability with the McCormick and Baxter site contaminated by creosote (>3.9 TU). Different sources of PAHs in the field, such as pyrogenic, petrogenic, and combustion-derived sources, need to be included separately to better assess the uncertainty of the ΣPAH model. Therefore, species-, compound-, and site-specific biases are unavoidable in

U.S. EPA ESGs for PAH mixtures if the ΣPAH model based on water-only LC50 TU is applied.

CONCLUSIONS

The ΣPAH model, based on Eq-P theory and sum of TUs calculated using water-only LC50, was developed to reduce the uncertainty in concentration–response relationships in field sediments, such as the bioavailability and PAH mixture toxicity. This approach is the same as that of the U.S. EPA for PAH mixtures in sediment evaluation. To our knowledge, this study is the first attempt to use water-only LC50 values to develop a ΣPAH model and apply it to freshwater sediments. The results suggest that the ΣPAH model includes species-specific (*Hyalella* sp. vs *Rhepoxynius* sp.) and chemical-specific (PAH vs DDT in *H. azteca*) biases, which are not incorporated in the Eq-P theory, as well as uncertainty from site-specific effects (creosote vs other PAH contamination sources) on bioavailability of sediment-associated PAHs.

In contrast, the relationship between the sum of TUs and the probability of toxicity could be analyzed and summarized by a logistic-regression model based on co-occurrence analysis. The sum of TUs corresponding to 50% probability of toxicity and its 95% CI can be used to assess the accuracy and precision of the ΣPAH model for field sediments. The logistic-regression model was useful for screening the toxicity of PAH mixtures in field sediment samples. However, results of this model reflect both the effects of co-occurring contaminants (i.e., nonmeasured PAHs and unknown pollutants) and the actual sediment exposure condition (i.e., overestimation of exposure compared to prediction by the Eq-P model). Thus, the result of the ΣPAH model based on water-only LC50 TU cannot be interpreted to reflect a cause–effect relationship. As alternatives with which to address a cause–effect relationship, approaches to reflect the actual exposure condition, such as using a site-specific sediment bioaccumulation factor and sum of TUs model based on species-specific CBRs, would allow development of a method to evaluate mixture effects based on the toxicity mechanism.

Acknowledgement—Primary funding for the database development was provided to the National Oceanic and Atmospheric Administration; the U.S. Environmental Protection Agency (EPA), Office of Research and Development; and the U.S. EPA, Office of Water under Interagency Agreements. Chris Ingersoll, Donald MacDonald, and Corinne Severn were instrumental in developing the database. This study was supported in part by the National Institute of Environmental Research, Ministry of Environment, Korea (Sediment Organic Compounds Bioassay Study 98-2000). GLERL contribution 1188.

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