Environ. Sci. Technol. **2002**, *36*, 3124–3130

Toxicokinetics and Time-Dependent PAH Toxicity in the Amphipod *Hyalella azteca*

JONG-HYEON LEE,[†] PETER F. LANDRUM,[*,‡] AND CHUL-HWAN KOH[†]

Department of Oceanography, Seoul National University, Seoul 151-742, Korea, and Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, 2205 Commonwealth Boulevard, Ann Arbor, Michigan 48105-1593

The relationship between toxicokinetics and time-dependent PAH toxicity to *Hyalella azteca* was examined to test the constant critical body residue (CBR) model. A constant CBR model is based on the assumption that the body residue for 50% mortality is constant for each PAH across exposure times. With a constant CBR, kinetic parameters determined through kinetic experiments would be similar to those estimated from time series toxicity data. Time-dependent toxicity was investigated using three types of data: time series LC_{50} data, $LT_{50}(c)$, and CBR values measured at multiple exposure times for live and dead animals. Kinetic parameters were measured independently. The constant CBR model did not predict the PAH toxicity time course for *H. azteca*. Since a first-order kinetic model predicted the bioaccumulation of the parent PAH except for naphthalene, this result is not due to a failure to predict the internal dose (body residue). The influence of metabolites on toxicity was negligible except for naphthalene. The LC_{50} values at multiple exposure times decreased to an incipient lethal concentration after *H. azteca* reached steady state. Measured CBR values also decreased with increasing exposure time. Thus, the time course of PAH toxicity is determined not only by the bioconcentration kinetics but also by the cumulative toxicity with increasing exposure time. Therefore, time-to-death or hazard models must be developed as a complement to toxicokinetic models to describe and predict the toxicity time course.

Introduction

About 60% of organic compounds in the environment exert a "baseline toxicity" referred to as narcosis (*1*, *2*). Polycyclic aromatic hydrocarbons (PAH) are considered to be typical nonpolar narcotic (anesthetic) compounds (*3*, *4*). According to the "narcosis hypothesis" for poorly metabolized nonpolar narcotic chemicals, a constant threshold exists for exposure concentration and time (*5*). The threshold for 50% mortality is often called the critical body residue (CBR) and is relatively constant (2–8 mmol/kg wet weight for acute mortality) and is also chemical and species independent for narcotic compounds (*6*).

* Corresponding author phone: (734)741-2276; fax: (734)741-2055; e-mail: landrum@glerl.noaa.gov.

† Seoul National University.

‡ National Oceanic and Atmospheric Administration.

On the basis of a first-order toxicokinetic model, the $LC_{50}(t)$ is given by

$$LC_{50}(t) = \frac{CBR}{(k_u/k_e)(1 - e^{-k_e t})} \quad (1)$$

where $LC_{50}(t)$ is the median lethal concentration at a given exposure time t, CBR is a constant body residue corresponding to 50% mortality (mmol kg^{-1}), k_u and k_e stand for the uptake clearance rate (L kg^{-1} h^{-1}) and the elimination rate constant (h^{-1}), respectively. In addition, the median lethal time ($LT_{50}(c)$) for a given exposure concentration c is given by

$$LT_{50}(c) = -(1/k_e) \ln (1 - LC_{50}(\infty)/c) \quad (2)$$

where $LC_{50}(\infty)$ is the incipient LC_{50} at infinite time (mmol L^{-1}), c is a water exposure concentration (mmol L^{-1}), and LT_{50} is the exposure time (h) for 50% mortality. The $LC_{50}(\infty)$ is equivalent to $CBR/(k_u/k_e)$, where k_u/k_e is equal to the bioconcentration factor (BCF) at steady state (L kg^{-1}).

The constant CBR model assumes that the response is immediate when the CBR is reached. In addition, kinetic rates must be unaffected by the onset of toxicity to make predictions of CBR. Furthermore, to apply this simple first-order toxicokinetic model, additional assumptions are required: (i) no change in toxicant bioavailability, (ii) no toxicant uptake from the diet, (iii) negligible biotransformation, and (iv) no growth or weight loss of the animal during the exposure (*7*).

With the constant CBR model, there is a temporal correlation between attaining steady state and attaining a stationary LC_{50} value. These time courses are ultimately governed by k_e. On the basis of the first-order toxicokinetic model (eqs 1 and 2), the kinetic parameter (k_e) was previously estimated from lethality bioassays using time variable $LC_{50}(t)$ data (*7*–*11*). Combining the first-order toxicokinetic model and the narcosis hypothesis for poorly metabolized nonpolar narcotic compounds, then toxicity-derived kinetics should yield kinetic values similar to those from bioconcentration experiments and body residue similar to the measured CBR values (*10*, *11*).

The CBR is a surrogate of the internal effective dose at the target site(s) for mortality in test animals. Such CBR values have been reported as tissue residues predicted from the incipient LC_{50} values and the toxicokinetics:

$$CBR = LC_{50}(\infty) \times BCF \quad (3)$$

with CBR equal to critical body residue in mmol kg^{-1}, $LC_{50}(\infty)$ equal to the incipient LC_{50} in mmol L^{-1}, and BCF equal to bioconcentration factor in L kg^{-1}. The BCF value is measured or calculated by k_u/k_e, which is estimated using live animals (*6*, *12*). These predicted CBR values have been compared with measured values known as the lethal body burden (LBB) (*5*, *13*, *14*). The LBB values are measured as body residue at the time of death. It has been assumed that the individual LBB values are constant and independent of both exposure level and duration. Thus, the LBB(t) values were presented as the average body residue of toxicant in dead animals from all of treatments at a given exposure time t and then were compared with the CBR values estimated by eq 3.

Another description relating LBB and toxicity, the mean lethal residue (MLR), is calculated as the average tissue

10.1021/es011201l CCC: $22.00 © 2002 American Chemical Society
Published on Web 06/07/2002

residue of all dead animals from each treatment level c_i [i.e., MLR(c_i)] (*15–17*). The MLR values are related temporally to the LT_{50} in each treatment level c_i [i.e., MLR($LT_{50}(c_i)$)]. Therefore, as many MLR values can be determined as the number of exposure concentrations in each toxicity experiment. Recently, a hypothetical concept was suggested to show the relationship between LBB and time-to-death within treatments and among treatments: A decrease in LBB with time-to-death was found when MLR of different treatments were compared, meanwhile within a treatment, LBB was found to increase with time-to-death (*18*).

However, in a sediment toxicity test or in the field, it is impossible to determine the body residue of dead animals because they degrade quickly. Thus, it is essential to develop a method to measure CBR against live animal concentrations. Assuming that the body residue in live animals and dead animals are similar (as supported below), the median lethal residue (LR_{50}) can be defined by the body residue in live animals at 50% mortality that corresponds to the LC_{50} value (*19–21*). The LR_{50} values are calculated by probit analysis using body residue data in live animals at a given termination time in toxicity experiment, e.g., 10 d. The above operational definitions for CBR (LBB, MLR, LR_{50}) have not been fully compared (*18, 21*), especially with respect to the time dependence of CBR values (*18*).

In the present study, bioconcentration, toxicity, and measured CBR data were used to address the question "Does the constant CBR model apply to PAH toxicity in the freshwater amphipod *H. azteca*?". The utility of the first-order toxicokinetic model was evaluated to predict the PAH bioconcentration process in *H. azteca* and the impact of PAH metabolism. The time-dependent LC_{50} values were predicted with a constant CBR model (eq 1) using measured k_e values and compared with measured $LC_{50}(t)$. The predicted CBR values were compared to measured values using three methods (LBB, MLR, and LR_{50}) to examine and analyze the time dependence of CBR. Finally, an approach is suggested for measuring the time-dependent CBR values to describe and predict the time course of toxicity.

Experimental Section

Experimental Design. For naphthalene, fluorene, phenanthrene, and pyrene, their $LC_{50}(t)$ and $LT_{50}(c)$ values were determined in three separate toxicity experiments for each compound using the freshwater amphipod *Hyalella azteca* exposed to aqueous concentrations of contaminants in a manner similar to Lotufo et al. (*23*). Toxicokinetic parameters (k_u and k_e) were measured at the same treatment levels. Since *H. azteca* is known to metabolize PAH, the percentages of metabolites for four PAHs were also analyzed. A detailed description of the experiments is given in the Supporting Information.

Modeling Bioaccumulation. The PAH toxicokinetics were analyzed using a first-order two-compartment model with water as the source and the organism as the recipient. Because the water concentrations declined until renewal, the assumption of constant water concentrations was invalid. Toxicokinetic parameters for uptake and loss were estimated by numerical integration of the following differential equation:

$$\frac{dR}{dt} = k_u(C_{t=t_i} - m_i t) - k_e R \qquad (4)$$

with R is the tissue residue in mmol kg^{-1}, $C_{t=t_i}$ is the time-dependent water concentrations in each treatment level i in mmol L^{-1}, k_u is the uptake clearance rate in L kg^{-1} h^{-1}, and k_e is the elimination rate constant in h^{-1}. The slope of the linear decline in water concentration, m_i, was specific for each water renewal interval and for each combination of treatment level and compound. For depuration, the water concentration was essentially zero (not measurable). The data were fit using the fourth-order Runga–Kutta approach with time step 0.01 in the software package Scientist (Version 2.01, MicroMath, Salt Lake City, UT).

Data Analysis for Toxicity Experiments. The LC_{50} at exposure time, t, was determined with TOXSTAT (version3.5, West Cheyenne, WY) using the trimmed Spearman–Karber method. The mortality data were not transformed because all of the percent mortality data showed normality of variance (χ^2 test, $p < 0.05$). Contaminant treatments were compared with the control treatment using the Dunnett's t-test. The significance level (α) was set at 0.05.

For the toxicity time dependency, the median lethal time for each treatment i ($LT_{50}(c_i)$) and the median lethal concentration for a given exposure time ($LC_{50}(t)$) were used. To calculate the $LT_{50}(c)$, toxicity data were used from all toxicity experiments (exp I, II, and III) for each compound. The LT_{50} values were calculated under the assumption of a linear relationship between ln (exposure time) and percent mortality.

Prediction of CBR with and without a Constant CBR Assumption. The constant CBR value was estimated from $LC_{50}(t)$ and the measured bioconcentration factor (BCF = k_u/k_e) using eq 3. The k_e values were also estimated directly from $LC_{50}(t)$ or $LT_{50}(c)$ data using eqs 1 and 2 and compared with the measured values.

In contrast, if CBR is not constant for exposure times, CBR values can be estimated from $LC_{50}(t)$ and BCF values according to the definition of CBR as follows:

$$LR_{50}(t) = (k_u/k_e) \times LC_{50}(t) \times (1 - e^{-k_e t}) \qquad (5)$$

where $LR_{50}(t)$ and $LC_{50}(t)$ are the median lethal residue (mmol kg^{-1}) and the median lethal concentration (mmol L^{-1}) to produce 50% mortality at a given exposure time t; k_u and k_e are the measured first-order kinetic parameters.

When a lethal toxicity occurs for 50% of test animals in each treatment level, c_i, the exposure time will be the same as the LT_{50} and the lethal body residue will be the mean lethal residue (MLR):

$$MLR(LT_{50}(c_i)) = (k_u/k_e)c_i(1 - e^{-k_e \times LT_{50}(c_i)}) \qquad (6)$$

where MLR($LT_{50}(c_i)$) is the average value of lethal residue for dead animals related to the median time-to-death (LT_{50}) in a given treatment level c_i (*16, 17*). The median lethal time (LT_{50}) is a function of exposure concentration (c_i). If body residues in dead animals are equal to those in live animals at 50% mortality within the same treatment level c_i, the MLR-($LT_{50}(c_i)$) would be equal to the $LR_{50}(t)$. The assumption of equal body residues at 50% mortality between live and dead organisms has been previously demonstrated (*22*). According to these definitions, the MLR can be calculated using $LT_{50}(c)$ and toxicokinetic parameters (k_u and k_e).

Comparison with Measured CBR Values. The predicted CBR values with and without the assumption of constant CBR were compared to three types of measured CBR values. (i) Measured LR_{50} values at day 10 (10-d LR_{50}) were calculated from body residue of the live animals at day 10 in toxicity test with the trimmed Spearman–Karber method (*19–21*). (ii) LBB($t_{d_{ij}}$) at each time-to-death $t_{d_{ij}}$ in each treatment level i were measured for individual animals j from all of treatment levels (*13*). (iii) Mean lethal residue (MLR) for dead animals were calculated using individual lethal values in each treatment level c_i and related to the median lethal time (LT_{50}) for each treatment level c_i [i.e., MLR($LT_{50}(c_i)$)] (*16, 17*).

TABLE 1. Kinetic Parameters (Uptake Clearance Rate k_u and Elimination Rate Constant k_e) and Bioconcentration Factor (BCF) of Fluorene, Phenanthrene, and Pyrene after Exposure to Various Water Concentrations[a]

compd	water concn (μg L^{-1})	$k_u \pm$ SD (L kg^{-1} h^{-1})	$k_e \pm$ SD (h^{-1})	r^2	T50[b] (h)	T95[c] (h)	BCF (k_u/k_e)
fluorene	295	249 ± 59	0.936 ± 0.233	0.848	0.7	3.2	266
	403	325 ± 99	1.276 ± 0.401	0.831	0.5	2.4	255
	532	236 ± 41	0.778 ± 0.140	0.904	0.9	3.9	303
	1055	276 ± 65	0.877 ± 0.216	0.856	0.8	3.4	315
phenanthrene	87	142 ± 11	0.323 ± 0.026	0.958	2.1	9.3	440
	144	170 ± 17	0.373 ± 0.039	0.934	1.9	8.0	456
	223	142 ± 14	0.300 ± 0.030	0.917	2.3	10.0	473
	343	139 ± 10	0.276 ± 0.020	0.957	2.5	10.9	504
pyrene	9.4	656 ± 51	0.127 ± 0.012	0.977	5.5	23.6	5165
	29.6	493 ± 46	0.130 ± 0.015	0.964	5.3	23.1	3792
	40.9	470 ± 48	0.126 ± 0.016	0.956	5.5	23.8	3730
	66.8	467 ± 30	0.201 ± 0.017	0.986	3.4	14.9	2323

[a] SD, standard deviation. [b] Elimination half-life. [c] The time to reach 95% of the steady state.

Results and Discussion

Experimental Conditions. The exposure conditions and survival information are provided in Table A1 in the Supporting Information. All tissue concentrations are provided on a wet weight basis.

Can a First-Order Kinetic Model Predict PAH Bioaccumulation in *H. azteca*? *Uptake, Elimination, and Biotransformation.* The measured body residues in the toxicokinetic experiments were well-fit by the first-order kinetic model with variable water concentrations (Table 1; Figure A1, Supporting Information). For fluorene, phenanthrene, and pyrene, the body residues attained 95% of steady state within 1 day. There was no significant difference in k_u and k_e values among treatment levels for each compound, although k_u values for pyrene and phenanthrene were lowest at the highest treatment level.

After 24-h exposure to fluorene (129 μg L^{-1}), phenanthrene (107 μg L^{-1}), and pyrene (9 μg L^{-1}), the mean (±SD) fractions of [^{14}C] activity in *H. azteca* tissues corresponding to metabolites were 16 ± 3%, 5 ± 2%, and 20 ± 7%, respectively. During a 4-d exposure to fluorene (374 μg L^{-1}) and pyrene (7 μg L^{-1}), the fraction of pyrene metabolites did not change (5 ± 2%, n = 3) while fluorene metabolism increased from 11% (day 1) to 22% (day 4, n = 3); however, the increase was not significant (p = 0.389). These metabolite levels were similar to those for anthracene (14% at 8-h exposure) and fluoranthene (17% at 24-h exposure) in *H. azteca* (*20, 26*).

There was no significant difference between BCF values measured at steady state (BCF_{SS}) for live amphipods and kinetic-based BCF values (k_u/k_e) for pyrene, phenanthrene, and fluorene (Figure 1). In contrast, measured BCF values for naphthalene were very high and deviated from the regression line. The steady-state body residues for naphthalene ranged from 0.98 to 47.7 mmol kg^{-1} wet wt based on total [^{14}C] activity. These body residues are much higher than CBR values reported for narcotic compounds (2-8 mmol kg^{-1} wet wt). Because of the relatively low toxicity at these elevated body residues, most [^{14}C] activity for naphthalene seems to be metabolites. BCF values for naphthalene and fluorene significantly decreased with increasing mortality (Table A1, Supporting Information). These compounds are the more readily metabolized of the PAH studied; thus, the exposure to a lethal concentration may affect the PAH toxicokinetics in *H. azteca* by decreasing the biotransformation rate particularly for easily metabolized PAH such as naphthalene. Biotransformation of PAH in *H. azteca* can alter the toxic response; however, k_u and k_e values were not affected by the extent of mortality (Table 1).

Toxicokinetic Parameters and BCF in Relation to K_{OW}. If naphthalene is excluded, the kinetic parameters exhibited significant relationships with log K_{OW}:

$$\log k_e = -0.800\ (\pm 0.080) \log K_{OW} + 3.258\ (\pm 0.371) \quad (r^2 = 0.910,\ p < 0.001,\ n = 12)$$

$$\log k_u = 0.336\ (\pm 0.139) \log K_{OW} - 2.122\ (\pm 0.648) \quad (r^2 = 0.369,\ p = 0.036,\ n = 12)$$

The measured and kinetic-based BCF values also yielded highly significant regressions with log K_{OW}:

measured BCF for live animals

$$\log \mathrm{BCF} = 1.303\ (\pm 0.078) \log K_{OW} - 2.994\ (\pm 0.350) \quad (r^2 = 0.934,\ p < 0.001,\ n = 22)$$

There was no significant difference in the accumulation of

measured BCF for dead animals

$$\log \mathrm{BCF} = 1.293\ (\pm 0.080) \log K_{OW} - 3.379\ (\pm 0.362) \quad (r^2 = 0.949,\ p < 0.001,\ n = 16)$$

kinetic-based BCF

$$\log \mathrm{BCF} = 1.136\ (\pm 0.096) \log K_{OW} - 2.380\ (\pm 0.450) \quad (r^2 = 0.933,\ p < 0.001,\ n = 12)$$

compounds in the dead organisms as compared to live animals considering the standard errors for the slopes and intercepts in two regression lines. This is critical when comparing CBRs by different measures. Thus, methods based on live organisms or dead organisms should yield similar values for a given level of mortality and confirms Hwang's (*22*) observation that the LR_{50} was the same whether calculated from the body residues of live or dead organisms.

BCF for nonpolar organic compounds in aquatic organisms usually exhibit a strong relationship with K_{OW} (*24*). The relationship proposed by Mackay (*24*) for BCFs in fish commonly is considered a benchmark for the equilibrium partitioning theory (*6, 25*). The measured BCF slopes for both live and dead animals are slightly steeper than the benchmark. Different intercepts in regression lines among different species generally reflect variations in lipid content. The regression equation for fish species has an estimated intercept of −1.32 (*24*) based on a mean lipid content of around 5% wet wt. Accounting for the relatively low lipid content (f_L = 1.8% wet wt base) of *H. azteca* (*23*), the kinetic-

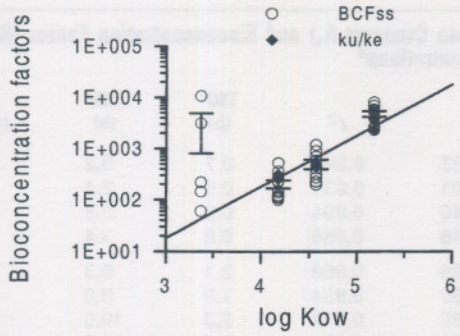

FIGURE 1. Relationship between log bioconcentration factor (BCF) vs log K_{OW} (octanol−water partition coefficient) for naphthalene, fluorene, phenanthrene, and pyrene in *H. azteca*. Apparent BCF values (BCF-live and BCF-dead) were calculated from live and dead animals in toxicity experiments (R/c, R = body residue, c = water exposure concentration). Kinetic-based BCF values were calculated as k_u/k_e from kinetic experiments using the same water concentration used for toxicity experiments. The solid line represents the regression line estimated using fluorene, phenanthrene, and pyrene data. The solid bars denote the standard deviation of the mean BCF values for individual PAH.

based BCF values were well fit by the benchmark equation (Figure 1):

$$\log \mathrm{BCF} = \log K_{\mathrm{OW}} + \log f_{\mathrm{L}}$$

$$= \log K_{\mathrm{OW}} - 1.745 \quad (r^2 = 0.919,\ n = 12)$$

Thus, for *H. azteca*, with the exception of naphthalene, the PAHs were in approximate equilibrium between lipid and water at steady state. These results are also in reasonable agreement with a first-order kinetic model with no biotransformation.

Can the Constant CBR Model Predict the Time Course of Toxicity of PAH in *H. azteca*? *Median Lethal Concentration* (LC_{50}). Mean ± SD control survival at termination of the experiment (day 10) was 100 ± 0% for naphthalene, 95 ± 9% for fluorene, 97 ± 3% for phenanthrene, and 95 ± 5% for pyrene. Survival at day 10 was significantly lower than the control at naphthalene concentrations of 1770 μg L^{-1}, fluorene concentrations of 478 μg L^{-1}, phenanthrene concentrations of 231 μg L^{-1}, and pyrene concentrations of 75 μg L^{-1} and greater (Table A1, Supporting Information). The 10-d LC_{50} values from exp I were lowest for pyrene having the highest hydrophobicity and highest for naphthalene having the lowest hydrophobicity with the relative magnitude among the values of approximately 1:4:8:34 (pyrene:phenantherene: fluorene:naphthalene) based on molar concentration. The variation among LC_{50} values was described by a linear relationship of log 10-d LC_{50} (mmol L^{-1}) vs log K_{OW} (log 10-d $LC_{50} = -0.973 \log K_{OW} + 1.560$, $r^2 = 0.995$; $p < 0.001$; $n = 6$).

Comparison between Measured and Predicted Toxicity Time Course, $LC_{50}(t)$. Because survival was monitored daily by nondestructive observation from replicate beakers, LC_{50} values at multiple exposure times could be calculated (Table A2. Supporting Information) and plotted versus exposure time in Figure 2. Body residues for all of the contaminants had approached steady state by at least day 2, but the LC_{50} values for all compounds continued to decrease after day 2 (Figure 2). Predicted LC_{50} values from the constant CBR model (eq 1) using the measured elimination rate constant (k_e) values did not fit the measured LC_{50} data. Furthermore, estimated k_e values (naphthalene, 0.071 ± 0.014; fluorene, 0.109 ± 0.060; phenanthrene, 0.020 ± 0.001; pyrene, 0.003 ± 0.004) from the constant CBR model (eq 1) using measured $LC_{50}(t)$ were much lower than the measured values (Table 1). Thus, the time course for PAH toxicity in *H. azteca* was not predicted

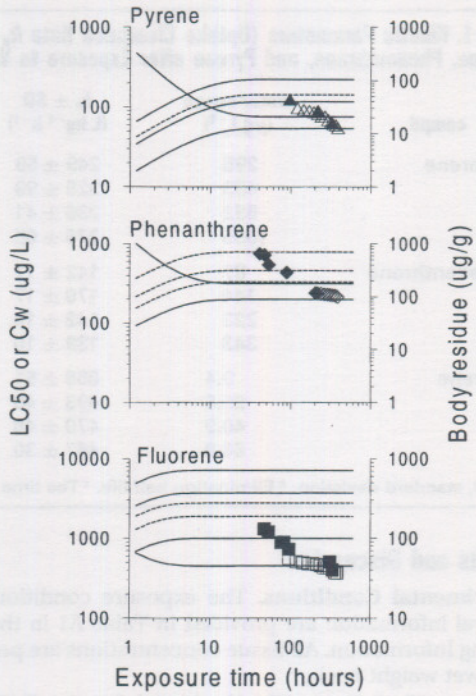

FIGURE 2. Plots of $LT_{50}(c)$ (closed symbols) and $LC_{50}(t)$ (open symbols) for fluorene, phenanthrene, and pyrene in *H. azteca* against the exposure period for body residue to attain the steady state. Dashed curves are body residues, and solid curves are the predicted $LC_{50}(t)$ from the constant CBR model.

by the constant CBR model, and time-to-death is a function not only of body residue but also of exposure time or factors related to exposure time.

In a similar study measuring $LC_{50}(t)$, kinetic parameters (k_u and k_e) and CBR for phentachlorophenol (PCP) in fathead minnows, the three independent data sets consistently fit a first-order kinetic model (*11*). These results suggest that a constant CBR model would be appropriate. In contrast, k_e values estimated from the whole body bioconcentration in rainbow trout were lower than those estimated from toxicity data for PCP (*10*). In this case, the k_e values in blood, liver, muscle, gills, and heart were larger than those in fat tissues indicating that the PCP kinetics at the site of toxic action were responsible for the effects rather than k_e in fat tissues, which dominates the overall toxicokinetics. Our measured k_e values from the kinetic experiments were greater than those estimated from the toxicity data. Considering the nonspecific toxicity mechanism of PAH as a narcotic, it is unexpected to have k_e values estimated from the toxicity data lower than those calculated from the kinetics. If the metabolites were toxic, the slow process of metabolite formation could be responsible for the continued decrease in $LC_{50}(t)$ after whole body residue achieved steady state. However, from the naphthalene data (Figure 3), it is clear that the toxicity of PAH metabolites in *H. azteca* are much lower than that of the parent compound. Therefore, PAH metabolism apparently acts as a detoxification process. This further supports the conclusion that in addition to toxicokinetic processes there is another time-limiting step affecting the toxicity time course of PAH in *H. azteca*.

Comparison between Predicted and Measured CBR Values. *Median Lethal Residue in Live Animals* (LR_{50}). Overall, survival declined with increasing treatment level for all the contaminants except naphthalene. This response was strongly associated with increasing body residues in the remaining live animals. In the case of naphthalene, body residue values presented in Figure 3 includes both parent naphthalene and its metabolites. The dose−response relationship for naph-

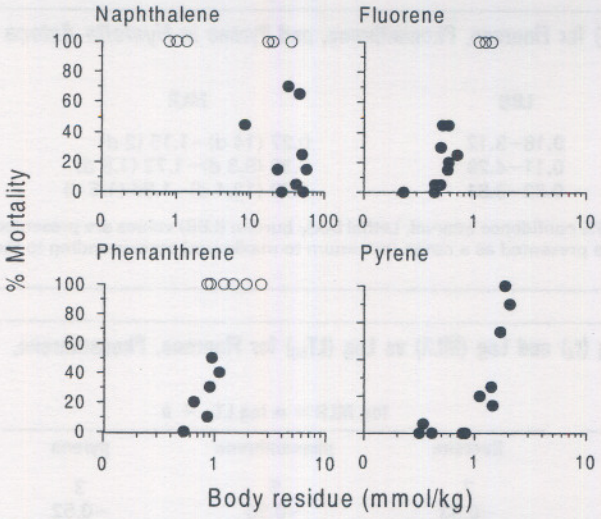

FIGURE 3. Body residue vs percent mortality in *H. azteca* after 10 d of exposure to a range of concentrations of naphthalene, fluorene, phenanthrene, and pyrene. Closed circles denote body residue at day 10. When all animals are dead at day 10, body residues at steady state before day 10 (open symbols) were used for calculation of LR_{50} values.

thalene was masked by the body residue of its metabolites, which are less toxic than the parent.

For other compounds except naphthalene, 10-d LR_{50} values were calculated using body residue of live animals in each treatment (Figure 3). To calculate 10-d LR_{50} values for fluorene and phenanthrene, body residues at steady state were used as a surrogate for body residue at day 10 for the 925 $\mu g\ L^{-1}$ fluorene and the 379 and 848 $\mu g\ L^{-1}$ phenanthrene concentrations. The 10-d LR_{50} values were 0.67 (95% CI, 0.65−0.71) mmol kg^{-1} for fluorene, 0.92 (0.89−0.95) mmol kg^{-1} for phenanthrene, and 1.66 (1.55−1.77) mmol kg^{-1} for pyrene (Figure 3). The LR_{50} values varied by about a factor of 2 and had a narrower range than the LC_{50} values by a factor of approximately 10.

LBB and MLR in Dead Animals. To investigate the influence of time-to-death on lethal body residue, the body residues were measured for individual dead amphipods (exp II and III) (Figure 4). The individual lethal residue or LBB ranged from 0.18 to 3.12 mmol kg^{-1} for fluorene, from 0.11 to 4.29 mmol kg^{-1} for phenanthrene, and from 0.22 to 3.81 mmol kg^{-1} for pyrene. In contrast, LBB of naphthalene had a wider range from 0.29 to 14.32 mmol kg^{-1} (Table A1, Supporting Information). The MLR values of fluorene in each treatment ranged from 0.27 ± 0.09 mmol kg^{-1} (LT_{50} = 14 d) to 1.15 ± 0.54 mmol kg^{-1} (LT_{50} = 2 d) (Table A1, Supporting Information). The MLR values of phenanthrene ranged from 0.38 ± 0.098 mmol kg^{-1} (LT_{50} = 8.3 d) to 1.72 ± 0.78 mmol kg^{-1} (LT_{50} = 1.8 d). The MLR values of pyrene ranged from 0.72 ± 0.51 mmol kg^{-1} (LT_{50} = 13.4 d) to 1.36 ± 0.37 mmol kg^{-1} (LT_{50} = 1.5 d) (Table A1, Supporting Information). Individual variations in lethal body residues within each treatment for all compounds were similar with CV values of 27−50% (fluorene), 7−71% (phenanthrene), and 39−55% (pyrene).

The measured LR_{50} and MLR values were slightly lower than those of *H. azteca* (1.1−5.6 mmol kg^{-1}) in other studies (*19, 26*) and also slightly lower than the range of CBR typically observed for nonpolar narcotics in other organisms (2−8 mmol kg^{-1}) (*6*). Compared with CBR values of PAH in other invertebrates and fish, the CBR values in this study were comparable to those in *L. plumulosus* (0.7−2.4 mmol kg^{-1}) (*27*) and copepods (1.2−2.7 mmol kg^{-1}) (*28*) but somewhat lower than those in *Diporea* spp. (2.7−9.4 mmol kg^{-1}) (*26, 29, 30*) and fathead minnows (8.0 mmol kg^{-1}) (*18*). On the basis of CBR, it appears that *H. azteca* is more sensitive to PAH than *Diporea* spp. and fathead minnows. However, after lipid normalization, the sensitivity of *H. azteca* (37−311 μmol g $lipid^{-1}$) is similar to those of *Diporea* spp. (39−134 μmol g $lipid^{-1}$) and fathead minnows (40−190 μmol g $lipid^{-1}$).

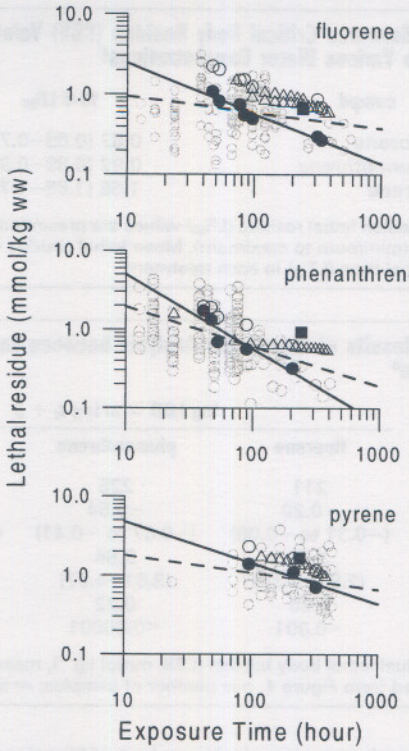

FIGURE 4. Comparison of measured and predicted CBR values of fluorene, phenanthrene, and pyrene in *H. azteca* for different exposure times. Measured CBR values include the individual lethal body burden (LBB) at the time-to-death (small open circles), the mean lethal residues (MLR) in dead animals at the median lethal time (LT_{50}) in each treatment (closed circles), and the10-d LR_{50} values for live animals (closed rectangles). Dashed lines denote the regression lines for the relationship between LBB and time-to-death; solid lines denote the regression lines for the relationship between MLR and LT_{50}. Predicted CBR values are $LR_{50}(t)$ values (open triangle) calculated from $LC_{50}(t)$, and MLR($LT_{50}(c)$) values (large open circle) calculated from $LT_{50}(c)$.

Time Dependence of Measured and Predicted CBR Values. Figure 4 shows a temporal variation of lethal body residue in dead animals for each compound. Individual LBB was significantly correlated with its time-to-death (t_d) using a power function (log LBB = m log $t_d + b$) (Table 3). For all compounds, MLR values declined with increasing exposure time. Neither the LBB or the MLR values matched the predicted CBR values from the constant CBR model. Predicted $LR_{50}(t)$ and MLR ($LT_{50}(c)$) using eqs 5 and 6 from $LC_{50}(t)$ and $LT_{50}(c)$ data showed a similar trend to measured MLR values. Differences between the measured and the predicted MLR and LR_{50} values are mainly due to the differences between BCF values calculated from dead animals or live animals and partially due to the small differences between the curves of $LT_{50}(c)$ and $LC_{50}(t)$ (Figure 2).

The time-dependent $LR_{50}(t)$ values predicted from $LC_{50}(t)$ values only assume a constant BCF among different treatment levels. Meanwhile, the constant CBR model requires an additional assumption of a constant threshold for toxicity (*9, 13*). The BCF did not change with increasing exposure concentration (Table 1), upholding the constant BCF assumption. Thus, the toxicity time course for PAH in *H. azteca* is regulated not only by kinetic process but also by cumulative interaction with a receptor, suggesting that there is another

TABLE 2. Measured Critical Body Residue (CBR) Values (mmol kg^{-1}) for Fluorene, Phenanthrene, and Pyrene in *Hyalella Azteca* Exposed to Various Water Concentrations[a]

compd	10-d LR_{50}	LBB	MLR
fluorene	0.67 (0.65−0.71)	0.18−3.12	0.27 (14 d)−1.15 (2 d)
phenanthrene	0.92 (0.89−0.95)	0.11−4.29	0.38 (8.3 d)−1.72 (1.8 d)
pyrene	1.66 (1.55−1.77)	0.22−3.81	0.72 (13.4 d)−1.36 (1.5 d)

[a] 10-d median lethal residue (LR_{50}) values are presented as mean and 95% confidence interval. Lethal body burden (LBB) values are presented as a range (minimum to maximum). Mean lethal residue (MLR) values are presented as a range (minimum to maximum) corresponding to the median lethal time (LT_{50}) in each treatment.

TABLE 3. Results of Regression Analysis between Log (LBB) vs Log (t_d) and Log (MLR) vs Log (LT_{50}) for Fluorene, Phenanthrene, and Pyrene[a]

	$\log LBB = m \log t_d + b$			$\log MLR = m \log LT_{50} + b$		
	fluorene	phenanthrene	pyrene	fluorene	phenanthrene	pyrene
n	211	225	120	7	5	3
m	−0.20	−0.54	−0.22	−0.63	−0.95	−0.52
	(−0.31 to −0.08)	(−0.67 to −0.41)	(−0.39 to −0.05)	(−0.80 to −0.46)	(−1.68 to −0.21)	(−3.15 to +2.11)
b	3.17	3.84	3.47	1.01	1.65	1.20
	(2.97−3.38)	(3.61−4.07)	(3.07−3.87)	(0.66−1.36)	(0.27−3.02)	(−4.82 to +7.21)
r^2	0.05	0.22	0.05	0.95	0.85	0.86
p value	<0.001	<0.00001	0.01	<0.001	0.02	0.24

[a] Individual lethal body burden (LBB, mmol kg^{-1}), mean lethal residue (MLR, mmol kg^{-1}), time-to-death (t_d), and the median lethal time (LT_{50}) are obtained from Figure 4. n is number of samples; m and b are constants presented with their 95% confidence interval.

time-dependent process independent of kinetics that affect the observed mortality response.

How Can We Measure and Estimate the Time-Dependent CBR? The toxicity time course is generally analyzed at a fixed mortality level, e.g., 50% mortality [i.e., $LC_{50}(t)$ or $LT_{50}(c)$]. This "iso-effective approach" was applied to the time-dependent CBR values (Figure 4). This analysis can be considered a "population-averaged approach". In this study, mean values of lethal body residue [$MLR(LT_{50}(c)$] were calculated and related to the median lethal time (LT_{50}) in each treatment c. The measured $LT_{50}(c)$ or $LC_{50}(t)$ and MLR-(LT_{50}) values showed a similar temporal pattern (Figures 2 and 4). The results, therefore, demonstrated that the longer the median time-to-death, the lower MLR value in each treatment on a population-averaged basis.

Several recent works, including the work reported here, show time-dependent CBR values for narcotic compounds (*14*, *18*) and for reactive and receptor-mediated compounds (*4*, *31*). It is notable that different approaches to study the time dependency of CBR produce different results (*18*). A decrease of CBR with time-to-death was found when the mean values of CBR at different treatments [$MLR(LT_{50}(c_i))$ in this study] were compared. In the same study (*18*), CBR within one treatment [$LBB(t_{d_i})$ in this study] was found to increase with time-to-death (Figure 4). These results are easily expected in accordance with the standard toxicity model such as log−logistic or probit model.

In the standard approach (log−logistic model or probit model) to determine LC_{50} values, individuals vary in physiological condition and, therefore, in threshold response values. The threshold response value of a particular individual is assumed to be a random trial from a bell-shaped frequency distribution, which leads to a sigmoid concentration−response curve for the number of survivors. The effect then is described deterministically at the level of the individual and stochastically at the level of the cohort of tested animals. If there is no variation of toxicokinetic parameters among individuals, individual LBBs would therefore increase along with time-to-death within a treatment (*18*). This would occur because the more tolerant an individual, the longer it survives, and the more contaminant the individual will accumulate, up to steady state. In contrast, if there is a variation in toxicokinetic parameters among individual organisms and this variation is independent of the variation of individual tolerance (*32*, *33*), individual variation of lethal residues measured from dead animals within a treatment would not necessarily increase along with time-to-death. More tolerant individuals may have lower residues than less tolerant individuals. Pawlisz and Peters (*33*) showed that mean body residues of the unaffected, immobilized, and dead *Daphnia* at a fixed exposure time differed significantly, but the cumulative curves overlapped. As a result, prediction of *Daphnia* responses from the body residue of toxicants is necessarily probabilistic. In this case, time-to-death curve would not be a function of tolerance but a function of individual variation of tolerance and kinetic parameters.

Since the animals used in a toxicity test are from the same population, the distributions of individual tolerance and toxicokinetic parameters can be assumed to be the same among treatments. This assumption is at least reasonable for toxicant with a nonspecific toxic mode of action such as narcotic compounds that cannot change the tolerance distribution in the population during the exposure period. Finally, although it is impossible to identify log time-to-death ($t_{d_{ij}}$) and log lethal residue (LR_{ij}) in the individual j level at each treatment level i, it is expected that the LR_i declined with increasing t_{d_i} at a given level of mortality with the same variance of the data points ($\log LR_{ij} - \log t_{d_{ij}}$) among different treatments.

In conclusion, it is clear that the toxicity time course of PAH in *H. azteca* is determined not only by bioconcentration kinetics but also by cumulative toxicity with increasing exposure time. Therefore, to describe and predict its time course, two different types of experiments need to be conducted using the same treatment levels at the same time: (i) toxicity experiments for gathering the time-to-death data at a given exposure level and (ii) toxicokinetic experiments to estimate kinetic parameters (k_u and k_e). In addition, these data need to be compared with the measured time-dependent CBR values to describe and predict the toxicity time course related to CBR. That seems to be the only method to relate the time course of toxicity in the manner of a dose−response relationship. Toxicity and bioconcentration experiments give us two types of information: toxicokinetics and toxico-

dynamics. Therefore, to describe and predict the time course of toxicity, toxicodynamic models such as modeling time-to-death or hazard modeling must be developed as well as toxicokinetic model.

Acknowledgments

This work was supported in part by the National Institute of Environmental Research (NIER), Ministry of Environment, Korea SORGBIOS 98-2000 (Sediment Organic Compounds Bioassay Study). This is GLERL Contribution No. 1233.

Supporting Information Available

Additional text, two tables, and one figure. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Vieth, G. D.; Call, D. J.; Brooke, L. T. *Can. J. Fish. Aquat. Sci.* **1983**, *40*, 743–748.
(2) Könnemann, H. *Toxicology* **1981**, *19*, 209–221.
(3) Herman, J. L. M. Quantitative Structure–Activity Relationships of Environmental Pollutants. In *Handbook of Environmental Chemistry. Vol. 2E. Reactions and Processes*; Hutzinger O., Ed.; Springer Verleg: Berlin, 1989; pp 111–162.
(4) Verhaar, H. J. M.; de Wolf, W.; Dyer, S.; Legierse, K. C. H. M.; Seinen, W.; Hermens, J. L. M. *Environ. Sci. Technol.* **1999**, *33*, 758–763.
(5) Van Hoogen, G.; Opperhuizen, M. *Environ. Toxicol. Chem.* **1988**, *7*, 213-219.
(6) McCarty, L. S.; Mackay, D. *Environ. Sci. Technol.* **1993**, *27*, 1719–1728.
(7) McCarty, L. S.; Ozburn, G. W.; Smith, A. D.; Bharath, A.; Orr, D.; Dixon, D. G. *Hydrobiologia* **1989**, *188/189*, 533–542.
(8) Mancini, J. L. *Water Res.* **1983**, *17*, 1355–1362.
(9) Chew, R. D.; Hamilton, M. A. *Trans. Am. Fish. Soc.* **1985**, *114*, 403–412.
(10) Van den Heuvel, M. R.; McCarty, L. S.; Lanno, R. P.; Hickie, B. E.; Dixon, D. G. *Aquat. Toxicol.* **1991**, *20*, 235–252.
(11) Hickie, B. E.; McCarty, L. S.; Dixon, D. G. *Environ. Toxicol. Chem.* **1995**, *14*, 2187–2197.
(12) McCarty, L. S. *Environ. Toxicol. Chem.* **1986**, *5*, 1071–1080.
(13) Sijm, D. T. H. M.; Schipper, M.; Opperhuizen, A. *Environ. Toxicol. Chem.* **1993**, *12*, 1117–1127.
(14) Van Wazel, A. P.; De Vries, D. A. M.; Kostense, S.; Sijm, D. T. H. M.; Opperhuizen, A. *Ecotoxicol. Environ. Saf.* **1995**, *28*, 53–70.
(15) Mortimer, M. R.; Connell, D. W. *Environ. Sci. Technol.* **1995**, *29*, 1881–1886.
(16) Chaisuksant, Y.; Yu, Q. M.; Connell, D. *Ecotoxicol. Environ. Saf.* **1997**, *37*, 66–75.
(17) Yu, Q.; Chaisuksant, Y.; Connell ,D. W. *Chemosphere* **1999**, *38*, 909–918.
(18) De Maagd, P. G. J.; Van de Klundert, I. C. M.; Van Wezel, A. P.; Opperhuizen, A.; Sijm, D. T. H. M. *Ecotoxicol. Environ. Saf.* **1997**, *38*, 232–237.
(19) Landrum, P. F.; Dupuis, W. S. Toxicity and Toxicokinetics of Pentachlorophenol and Carbaryl to *Pontoporeia hoyi* and *Mysis relicta*. In *Aquatic Toxicology and Risk Assessment: Thirteenth Volume;* Landis W. G., Vand Der Schalie W. H., Eds.; STP 1096; ASTM: Philadelphia PA, 1990; pp 278–289.
(20) Kane Driscoll, S.; Landrum, P. F. *Environ. Toxicol. Chem.* **1997**, *16*, 2179–2186.
(21) Fisher, S. W.; Chordas, S. W., III; Landrum, P. F. *Aquat. Toxicol.* **1999**, *45*, 115–126.
(22) Hwang, H. Contaminant body residues in *Chironomus riparius* to assess acute and chronic toxic effects of environmental contaminants. Ph.D. Thesis, The Ohio State University, Columbus, OH, 2000, p 62.
(23) Lotufo, G. R.; Landrum, P. F.; Gedeon, M. L.; Tigue, E. A.; Herche, L. R. *Environ. Toxicol. Chem.* **2000**, *19*, 368–379.
(24) Mackay, D. *Environ. Sci. Technol.* **1982**, *16*, 274–278.
(25) McCarty, L. S.; Mackay, D.; Smith, A. D.; Ozburn, G. W.; Dixon, D. G. *Environ. Toxicol. Chem.* **1992**, *11*, 917–930.
(26) Kane Driscoll, S.; Harkey, G. A.; Landrum, P. F. *Environ. Toxicol. Chem.* **1997**, *16*, 754–761.
(27) Kane Driscoll, S.; Schaffner L. C.; Dickhut R. M. *Mar. Environ. Res.* **1998**, 45, 269–284.
(28) Lotufo, G. R. *Aquat. Toxicol.* **1998**, *44*, 17–30.
(29) Landrum, P. F.; Dupuis, W. S.; Kukkonen, J. *Environ. Toxicol. Chem.* **1994**, *13*, 1769–1780.
(30) Landrum, P. F.; Eadie, B. J.; Faust, W. R. *Environ. Toxicol. Chem.* **1991**, *10*, 35–46.
(31) Legierse, K. C. H. M.; Verhaar, H. J. M.; Vaes, W. H. J.; Wouter, H. J.; De Bruijn, J. H. M.; Hermens, J. L. M. *Environ. Sci. Technol.* **1999**, *33*, 917–925.
(32) Bedaux, J. J. M.; Kooijman, S. A. L. M. Stochasticity in Deterministic Model. In *Handbook of Statistics Vol. 12: Environmental Statistics*; Rao, C. R., Patil, G. P., Ross, N. P., Eds.; Elsevier Science B.V.: Amsterdam, 1994; pp 561–581.
(33) Pawlisz, A. V.; Peters, R. H. *Environ. Sci. Technol.* **1993**, *27*, 2795–2800.

Received for review August 15, 2001. Revised manuscript received April 19, 2002. Accepted April 23, 2002.

ES011201L